Risk Assessment of Hexabromocyclododecane

[CASRN 3194-55-6]

Supplemental Information on Human Health Hazard

Acknowledgment of ORD-NCEA

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1. Detailed Hazard Overview

1.1. Thyroid Effects

1.1.1. Human Evidence

The association between HBCD exposure and alterations of thyroid hormones was investigated in populations at different lifestages. Specifically, investigations of the potential effects of HBCD on the thyroid in humans have been conducted in infants and children participating in birth cohort studies in the Netherlands {Roze, 2009, 758049} and Norway {Eggesbø, 2011, 787656}, adolescents participating in a cross-sectional general population study in areas around industrial sites in Belgium {Kiciński, 2012, 1927571}, and adult men attending an infertility clinic in the United States (cross-sectional study) {Johnson, 2013, 1676758}. In addition, there is one case-control study of hypothyroidism in Korean mother and infant pairs {Kim, 2014, 2324769}. Of these five studies, only two were large scale (>500 participants) {Eggesbø, 2011, 787656;Kiciński, 2012, 1927571}, and only one included an analysis that allowed for the examination of exposure-response patterns {Eggesbø, 2011, 787656}. Quantitative methods used by several of the studies resulted in 25–75% of samples below stated detection limits {Eggesbø, 2011, 787656;Kiciński, 2012, 1927571;Kim, 2014, 2324769}. While some of the available studies included consideration of other suspected thyroid-disrupting chemicals, none considered known thyroid antagonists such as perchlorate, thiocyanate, or nitrate {Tonacchera, 2004, 757426;Steinmaus, 2013, 3042121}. Other study limitations and a summary of overall confidence in the results are noted in [REF _Ref532801962 \h * MERGEFORMAT]. Studies are ordered by the age at outcome evaluation, and then by overall confidence in the study.

A Norwegian birth cohort did not find a statistically significant association between the levels of HBCD measured in breast milk and thyroid-stimulating hormone (TSH) levels in newborns {Eggesbø, 2011, 787656}. Elevated, but non-statistically significant, odds ratios (range: 1.3–1.6) were reported for increased TSH in relation to increasing HBCD levels in breast milk that are suggestive of a potential association; however, confidence intervals (CIs) around each of the point estimates were relatively wide (based on approximately 30 individuals per group) and a clear dose-response was not observed. This analysis controlled for several potential mediators of normal thyroid hormone variability and several thyroid disruptors (e.g., polychlorinated biphenyls [PCBs], polybrominated diphenyl ethers [PBDEs], and hexachlorobenzene). Adjustments for iodine deficiency were not made; however, the study authors noted that this condition is rare in Norway {Eggesbø, 2011, 787656}.

A study in adolescents ages 13–17 years who lived in areas around industrial sites in Belgium (n = 515) did not find an association between serum concentrations of HBCD and concurrent measures of TSH, thyroxine (T4), or triiodothyronine (T3) {Kiciński, 2012, 1927571}. Since approximately 75% of serum concentrations were below the limit of quantitation (LOQ), analyses were dichotomized to compare effects associated with HBCD concentrations above and below the LOQ. The three remaining studies {Roze, 2009, 758049;Kim, 2014, 2324769;Johnson, 2013, 1676758} had reporting deficiencies that limit the ability to interpret results from these studies (Table 1-2). In studies of infants {Roze, 2009, 758049} and adult men {Johnson, 2013, 1676758}, the authors did not identify a statistically significant

relationship between HBCD and a specific thyroid hormone; quantitative results pertaining to the magnitude or direction of association between HBCD and thyroid hormones were not reported. {Kim, 2014, 2324769@@author-year} found no significant correlations between α-, β-, or γ-HBCD and any thyroid hormones in infants with congenital hypothyroidism; however, reporting limitations of this case-control study (i.e., no information on participant recruitment) and analysis (i.e., 25% of samples were below the limit of detection [LOD]) were noted.

Overall, the human database for HBCD is inadequate to support conclusions regarding the relationship between HBCD exposure and thyroid effects. The studies of HBCD exposure in relation to variation in thyroid hormone levels or thyroid disease (congenital hypothyroidism) do not provide a basis for assessing a causal association at any lifestage.

1.1.2. Animal Evidence

Several short-term and subchronic rodent studies evaluated the effects of HBCD on the thyroid, specifically serum thyroid hormone levels, thyroid histopathology, and thyroid weight. Two of these studies investigated thyroid-related endpoints at time-points approximately 4–8 weeks following the end of dosing {WIL Research, 2001, 787787;Saegusa, 2009, 787721}. The evidence pertaining to thyroid effects in experimental animals following oral exposure to HBCD is summarized in [REF _Ref532802070 \h * MERGEFORMAT] and [REF _Ref532802191 \h * MERGEFORMAT]. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. If not otherwise indicated, endpoint measurements were made in adults.

1.1.3. Thyroid hormones

Several studies in rats reported HBCD-related effects on thyroid hormone levels using radioimmunoassay {van der Ven, 2009, 589273;van der Ven, 2006, 787745;Ema, 2008, 787657} or electrochemiluminescence immunoassay {Saegusa, 2009, 787721;WIL Research, 2001, 787787}.

TSH levels were generally increased in most dosed groups (male and female F0 and F1 CD rats {Ema, 2008, 787657}, male and female CD rats {WIL Research, 2001, 787787}, and male weanling CD rats {Saegusa, 2009, 787721}. These increases reached statistical significance in male weanlings (postnatal day [PND] 20) {Saegusa, 2009, 787721} and female adult rats (F0 and F1) {Ema, 2008, 787657}. Additional support for HBCD-mediated increases in TSH are provided by {van der Ven, 2006, 787745@@author-year}; although serum TSH levels were not directly measured, female rats exposed to 200 mg/kg-day HBCD for 28 days showed a statistically significant increase in pituitary TSH immunostaining, suggesting elevated synthesis and release of this hormone.

Statistically significant decreases in T4 (up to −38% of control) were observed in F0 rats exposed to approximately 1,000–1,300 mg/kg-day HBCD {Ema, 2008, 787657}. A dose-related decrease in T4 was also observed in the F1 generation, with a 28% decrease in T4 in high-dose females {Ema, 2008, 787657}. Similarly, male and female rats exposed for 90 days to doses up to 1000 mg/kg-day were observed to have a dose-related decrease in T4 (up to -37% of control) {WIL Research, 2001, 787787}. Adult female rats exposed to up to 200 mg/kg-day HBCD for 28 days also showed a significant dose-dependent decrease in serum T4 (26% decrease at 200 mg/kg-day) {van der Ven, 2006, 787745}; a dose-

related decrease was not observed in male rats in the same study. The available developmental and one-generation toxicity studies did not detect alterations in levels of T4 in offspring at maternal doses ranging from approximately 100 to 1,500 mg/kg-day {Saegusa, 2009, 787721;van der Ven, 2009, 589273}. Serum levels of T3 were also investigated in several studies {van der Ven, 2009, 589273;Ema, 2008, 787657;van der Ven, 2006, 787745;Saegusa, 2009, 787721; WIL Research, 2001, 787787}, but only one detected a statistically significant effect. A 15% decrease in T3 levels relative to controls was observed in male weanling rats treated gestationally and lactationally at maternal doses of 1,505 mg/kg-day {Saegusa, 2009, 787721}.

The pattern of increased TSH and decreased T4 observed in the two-generation reproductive study {Ema, 2008, 787657} is consistent with the multi-loop feedback system of the hypothalamus-pituitary-thyroid (HPT) axis {Fisher, 2012, 3042123}. The same patterns of effect in TSH and T4 were reported by {WIL Research, 2001, 787787@@author-year}; however, confidence in the hormone measurements from this study is low because approximately 50% of control samples used for TSH measurements were below the limit of detection and the remaining samples were 1–2 orders of magnitude lower than controls in other available studies, calling into question the conduct of the assay.

Two studies also measured thyroid hormone levels 4 weeks {WIL Research, 2001, 787787} or 8 weeks {Saegusa, 2009, 787721} after the end of dosing. Treatment-related changes in TSH and T3 levels were still present 8 weeks after the end of dosing in developmentally-exposed rats; however, the change was statistically significant for T3 only {Saegusa, 2009, 787721}. In contrast, T4 and TSH levels in rats exposed as adults returned to control levels within 4 weeks after cessation of exposure {WIL Research, 2001, 787787}.

1.1.4. Thyroid histopathology

Histopathological changes indicative of thyroid activation were observed in some studies in experimental animals following exposure to HBCD. A 28-day study using doses up to 200 mg/kg-day qualitatively reported a dose-dependent increase in thyroid activation (i.e., follicle size, epithelial cell height, vacuolization, and nuclear size) in both male and female adult rats { van der Ven, 2006, 787745}. A dose-related increase in the incidence of thyroid follicular cell hypertrophy was reported in adult male and female rats exposed to HBCD for 90 days and in female rats developmentally exposed to approximately 1,000–1,500 mg/kg-day for 30 days {WIL Research, 2001, 787787;Saegusa, 2009, 787721}. A similar dose-related effect was not observed in a 28-day study at doses up to 1,000 mg/kg-day {WIL Research, 1997, 787758} or in a two-generation reproductive toxicity study at doses up to approximately 1,300 mg/kg-day {Ema, 2008, 787657}. A statistically significant increase (46–87%) in the incidence of small thyroid follicles was reported in both F0 and F1 high-dose animals in a two-generation reproductive toxicity study {Ema, 2008, 787657}. This histological observation is likely indicative of a loss of colloid, which functions as a reservoir from which T3 and T4 can be released into the bloodstream as needed. With long-term TSH elevation, endocytosis of colloid occurs faster than synthesis, resulting in the progressive depletion of colloid and decreased follicle size {Rosol, 2013, 3042122}. Female mice exposed to approximately 200 mg/kg-day HBCD for 28 days showed a 20 and

26% decrease in follicle and colloid areas, respectively; however, this change did not reach statistical significance {Maranghi, 2013, 1927558}.

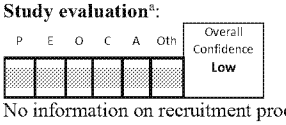
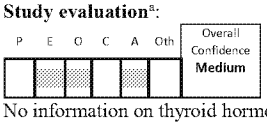
1.1.5. Thyroid weight

Several studies in rats reported treatment-related increases in thyroid weight {van der Ven, 2006, 787745;Saegusa, 2009, 787721;WIL Research, 2001, 787787;Ema, 2008, 787657}; however, the response patterns were not consistently dose-related nor were responses consistent across sexes. In animals exposed as adults only, several studies reported increased relative thyroid weights in female rats at doses ranging from approximately 30 to 1,500 mg/kg-day HBCD {van der Ven, 2006, 787745;Saegusa, 2009, 787721;WIL Research, 2001, 787787;Ema, 2008, 787657}, whereas only one study reported the same effect in males exposed to approximately 1,000 mg/kg-day {Ema, 2008, 787657}. In animals exposed to HBCD during development, statistically significant increases in thyroid weight were observed in male and female F1 adults exposed to 1,142 and 1,363 mg/kg-day, respectively {Ema, 2008, 787657} and adult males, but not females, 8 weeks after gestational and lactational exposure to ≥146 mg/kg-day {Saegusa, 2009, 787721}. In a one-generation reproductive study, no changes in absolute thyroid weight were reported in male or female F1 rats at doses up to 100 mg/kg-day {van der Ven, 2009, 589273}; relative thyroid weight was not reported.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Evidence pertaining to thyroid effects in humans following exposure to HBCD

Reference and study design	Results		
Studies in infants			
{Eggesbø, 2011, 787656@@author-year} (Norway, 2003–2006) **Population:** Birth cohort, recruited within 2 wks of delivery (able and willing to provide breast milk sample), 396 randomly selected for analysis; 239 of these were after February 2004 when the link to the thyroid screening data became available; 193 with HBCD data (46% girls) **Exposure measures: B**reast milk, collected at a median of 33 d after delivery (samples pooled over 8 consecutive mornings) Total HBCD detected in 67.9% of samples LOQ = 0.2 ng/g lipid Median 0.54 (range: 0.1–31) ng/g lipid **Effect measures:** TSH (whole blood spots) measured in infants 3 d after delivery (linked data beginning in February 2004); immunoassay (clinical lab) **Analysis:** Linear regression for ln TSH (continuous) and logistic regression for dichotomized ln TSH (at 80th percentile); see results column for consideration of covariates. Referent category includes all samples less than the LOQ (n = 62, 32%); remainder of population divided into four equally-sized categories.	Association between HBCD level in breast milk with neonatal TSH levels:		
	Exposure category (ng/g lipid) (N)	Adjusted beta for ln TSH (95% CI)[b]	Adjusted odds ratio for TSH ≥80th percentile (95% CI)[c]
	0.10 (62)	*(Referent)*	*(Referent)*
	0.13–0.52 (31)	−0.01 (−0.21, 0.20)	1.3 (0.3, 4.5)
	0.53–0.79 (33)	0.02 (−0.18, 0.22)	1.4 (0.3, 6.1)
	0.80–1.24 (33)	0.12 (−0.08, 0.33)	1.6 (0.4, 6.1)
	1.29–31.2 (34)	0.03 (−0.17, 0.23)	1.3 (0.3, 5.8)
	Per interquartile range increase:	−0.00 (−0.02, 0.02)	1.0 (0.8, 1.1)
	Adjusted for age at TSH screening, maternal BMI, county, p,p-DDE, hexachlorobenzene, delivery type, pregnancy preeclampsia, and hypertension. Also evaluated but eliminated were maternal education, age at delivery, Norwegian nationality, season, parity, smoking, sex, gestational age, beta-hexachlorocyclohexane, oxychlordane, and sum of all PCB congeners. EPA has lower confidence in results per interquartile range		

Reference and study design	Results
Study evaluation[a]: [EMBED PBrush]	increase than in categorical analysis; this analysis used HBCD as a continuous variable. The inclusion of non-detects in this analysis presents considerable uncertainty in the interpretation of the results.
{Roze, 2009, 758049@@author-year} (the Netherlands, COMPARE cohort, 2001–2002) **Population:** Birth cohort, 90 singleton, term births, 62 of 69 (90%) mother-child pairs randomly selected from the cohort for HBCD measures in serum **Exposure measures:** Prenatal exposure, maternal serum at 35th week of pregnancy 1,2,5,6,9,10-HBCD (HBCD) detected in all samples LOD 0.8 pg/g serum Median 0.8 (range: 0.3–7.5) ng/g lipids **Effect measures:** Thyroid hormones (cord blood samples, n = 51, selected based on amount of sample available): T4, free T4, reverse T3, T3, TSH, throxine-binding globulin (assay not described) **Analysis:** Pearson correlation (for normally distributed variables) or Spearman's rank correlation (for non-normally distributed variables) **Study evaluation**[a]: [EMBED PBrush] No information on thyroid hormone assays; limited analysis and inadequate reporting of results; small sample size	Results for correlations between HBCD and cord blood thyroid hormone levels were not shown, but were stated to be not statistically significant.
{Kim, 2014, 2324769@@author-year} (South Korea, 2009–2010) **Population:** 26 infants with congenital hypothyroidism and their mothers, 12 healthy infant-mother pairs from the same hospital department also collected (case-control). Age of infants 1–24 mo; most 1–3 mo; excluded obese mothers (normal group only). Sex of infants not reported. **Exposure measures:** Serum, α, β, γ-HBCD, most samples collected 1–3 mo after birth, samples from two congenital hypothyroidism infants collected 18 and 24 mo after birth LOQ 0.036 ng/g lipid (% less than detection limit not reported) Total HBCD: Mean 8.55 ng/g lipid, range from less than method detection limit to 166 ng/g lipid **Effect measures:** Congenital hypothyroidism (not defined) **Analysis:** Two-sided student t-tests; comparisons between mothers of cases and controls, and between infant cases and controls. Values below LOQ replaced by a value of 0.5 times the LOQ; concentration data normalized, excluding outliers (not defined), to sum of PBDEs, HBCDs, and tetrabromobisphenol A.	Congenital hypothyroidism — Healthy controls Mothers, mean HBCD level (SD) α-HBCD: 0.494 (1.52) — 2.57 (1.48)* β-HBCD: 0.27 (0.933) — 0.461 (1.08) γ-HBCD: 2.72 (1.42) — 8.86 (2.81) Infants, mean HBCD level (SD) α-HBCD: 2.42 (3.33) — 1.84 (2.5) β-HBCD: 0.578 (1.71) — 0.462 (0.768) γ-HBCD: 5.16 (2.42) — 14.05 (2.87)

Reference and study design	Results
Study evaluation[a]:  No information on recruitment process for cases or controls; 2 of the 26 cases who were ages 18 and 24 mo; approximately 25% less than the LOD; uncertain impact of exclusion of outliers	
Studies in adolescents	
{Kiciński, 2012, 1927571@@author-year} (Belgium, 2008–2011) **Population:** 515 adolescents (13–17 yrs old) from two industrial sites and randomly selected from the general population; participation rates 22–34% in the three groups, sample size varied by test **Exposure measures:** Serum samples, HBCD >75% were less than the LOQ (LOQ = 30 ng/L); Median <30 (range: <LOQ–234) ng/L **Effect measures:** Thyroid hormones: Free T3, free T4, TSH (immunoassay not described) **Analysis:** Regression models (linear or negative binomial depending on outcome); HBCD dichotomized **Study evaluation**[a]:  No information on thyroid hormone assays; 75% of HBCD less than the LOD (dichotomized analysis)	Thyroid hormone results (estimated from Figure 4 of {Kiciński, 2012, 1927571@@author-year}: Beta (95% CI)[d] Free T3 (pg/mL) 0.08 (−0.08, 2.3) FreeT4 (mg/dL) −0.02 (−0.03, 0.09) TSH (%) 0.0 (−4, 13) Linear regression models for free T3 and free T4; negative binomial model for TSH. All models adjusted for age, gender, blood lipids, and BMI. Additional covariates evaluated included smoking, parental smoking, parental education, and parental home ownership, physical activity, computer use, alcohol and fish consumption, blood lead, and blood PCBs, and were included based on a stepwise regression procedure.
Studies in adult men	
{Johnson, 2013, 1676758@@author-year} (United States, 2002–2003) **Population:** 38 men (18–54 yrs old), from couples seeking infertility treatment; approximately 65% participation into general study; participation rate in the vacuum bag collection phase of the study not reported **Exposure measures:** HBCD exposure from vacuum bag dust; three main stereoisomers of HBCD presented together HBCD detected in 97% of samples; LOD not reported; median 246 ng/g dust (90th percentile 1,103 ng/g dust) **Effect measures:** Non-fasting blood sample {immunoassay details in \Meeker, 2008, 2238550} TSH free T4 free T3 **Analysis:** All variables analyzed as continuous variables; Spearman's correlation between HBCD in	Adjustment for age and BMI produced similar results to the bivariate results (data not reported). No statistically significant changes in thyroid hormones (result not shown).

Reference and study design	Results
house dust and serum hormone levels; multivariable models adjusted for age and BMI **Study evaluation**[a]: [EMBED PBrush] Limited analysis and inadequate reporting of results; small sample size	

*p = 0.004; unadjusted for age and sex.
[a]Evaluation of sources of bias or study limitations (see Systematic Review Methods/Epidemiology Studies, and Appendix B, Table B-3): P = population selection; E = exposure misclassification; O = outcome misclassification; C = confounding; A = analysis; Oth = other feature affecting interpretation of results. Extent of column shading reflects degree of limitation.
[b]0.0 = no association.
[c]1.0 = no association.
[d]Beta is for HBCD >30 ng/L (LOQ) versus <30 ng/L; 0.0 = no association.

BMI = body mass index; EPA = U.S. Environmental Protection Agency; SD = standard deviation

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Evidence pertaining to thyroid effects in animals following exposure to HBCD

Reference and study design	Results			
Serum thyroid hormones				
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation Thyroid hormones were measured by radioimmunoassay in adults only	**Doses** (mg/kg-d)			
	Male, F0	**0**	**10**	**101**	**1,008**
	Female, F0	**0**	**14**	**141**	**1,363**
	Male, F1	**0**	**11**	**115**	**1,142**
	Female, F1	**0**	**14**	**138**	**1,363**
	TSH (ng/mL)				
	Male, F0 (n = 8)				
	Mean (SD)	16.15 (3.78)	16.18 (8.61)	19.14 (6.02)	23.26 (10.90)
	% of control[a]	–	0%	19%	44%
	Female, F0 (n = 8)				
	Mean (SD)	10.68 (1.35)	14.83* (2.47)	15.37* (2.17)	21.59* (8.87)
	% of control[a]	–	39%	44%	102%
	Male, F1 (n = 8)				
	Mean (SD)	11.93 (4.62)	11.50 (2.94)	15.78 (6.48)	15.54 (5.76)
	% of control[a]	–	−4%	32%	30%
	Female, F1 (n = 8)				
	Mean (SD)	10.35 (2.04)	15.36 (4.18)	18.09* (5.23)	17.28* (5.58)
	% of control[a]	–	48%	75%	67%
	T4 (µg/dL)				
	Male, F0 (n = 8)				
	Mean (SD)	4.04 (1.42)	3.98 (0.89)	2.97 (0.76)	2.49* (0.59)
	% of control[a]	–	−1%	−26%	−38%

Reference and study design	Results							
	Female, F0 (n = 8)							
	Mean (SD)	2.84 (0.61)		3.14 (0.48)		3.00 (0.77)		1.96* (0.55)
	% of control[a]	–		11%		6%		−31%
	Male, F1 (n = 8)							
	Mean (SD)	3.54 (0.29)		3.44 (0.86)		3.32 (0.98)		3.18 (0.48)
	% of control[a]	–		−3%		−6%		−10%
	Female, F1 (n = 8)							
	Mean (SD)	3.59 (1.08)		3.56 (0.53)		3.39 (1.21)		2.58 (0.37)
	% of control[a]	–		−1%		−6%		−28%
	T3 (ng/dL)							
	Male, F0 (n = 8)							
	Mean (SD)	143.6 (29.0)		138.2 (21.6)		121.6 (15.6)		126.9 (16.3)
	% of control[a]	–		−4%		−15%		−12%
	Female, F0 (n = 8)							
	Mean (SD)	133.1 (15.9)		140.9 (16.3)		146.5 (29.5)		134.7 (25.6)
	% of control[a]	–		6%		10%		1%
	Male, F1 (n = 8)							
	Mean (SD)	122.1 (9.9)		123 (13.7)		123.6 (22.6)		122.3 (20.4)
	% of control[a]	–		1%		1%		0%
	Female, F1 (n = 8)							
	Mean (SD)	146.7 (17.5)		143.3 (18.1)		132.1 (26.2)		130.4 (17.8)
	% of control[a]	–		−2%		−10%		−11%
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Thyroid hormones (total T3/T4) were measured by radioimmunoassay in adults only	**Doses** (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	T4 (nmol/L)								
	Male, F0 (n = 5)[b]								
	Mean (SD)	62.0 (4.7)	–	–	–	–	–	–	54.2 (13.8)
	% of control[a]	–	–	–	–	–	–	–	−13%
	Female, F0 (n = 5)[b]								
	Mean (SD)	44.4 (9.3)	–	–	–	–	–	–	38.0 (17.6)
	% of control[a]	–	–	–	–	–	–	–	−14%
	Male, F1 (n = 3−5)								
	Mean (SD)	44.8 (4.55)	48.6 (7.6)	46.3 (8.2)	47.2 (3.4)	42.6 (6.6)	45.0 (4.3)	46.6 (5.1)	47.6 (12.4)
	% of control[a]	–	8%	3%	5%	−5%	0%	4%	6%
	Female, F1 (n = 3−5)								
	Mean (SD)	50.6 (16.6)	37.8 (13.4)	38.8 (8.2)	49.6 (11.1)	44.8 (13.5)	59.7 (4.9)	41.4 (12.1)	47.0 (10.8)
	% of control[a]	–	−25%	−23%	−2%	−11%	18%	−18%	−7%
	T3 (nmol/L)								
	Male, F0 (n = 5)[b]								

Reference and study design	Results							
	Mean (SD)	0.9 (0.1)	--	--	--	--	--	--	0.8 (0.1)
	% of control[a]	--	--	--	--	--	--	--	−11%
	Female, F0 (n = 5)[b]								
	Mean (SD)	0.8 (0.2)	--	--	--	--	--	--	0.9 (0.3)
	% of control[a]	--	--	--	--	--	--	--	12%
	Male, F1 (n = 3−5)								
	Mean (SD)	0.9 (0.1)	1.2 (0.2)	1.0 (0.1)	1.0 (0.1)	1.0 (0.1)	0.9 (0.1)	0.9 (0.1)	1.0 (0.1)
	% of control[a]	--	33%	11%	11%	11%	0%	0%	11%
	Female, F1 (n = 3−5)								
	Mean (SD)	1.1 (0.3)	1.2 (0.2)	1.1 (0.2)	1.1 (0.1)	1.2 (0.2)	1.4 (0.1)	1.0 (0.1)	1.0 (0.1)
	% of control[a]	--	9%	0%	0%	9%	27%	−9%	−9%
{WIL Research, 2001, 787787@@author-year} Rats, Crl:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown Thyroid hormones (total T3/T4) measured by electro-chemiluminescence immunoassay in adults only	**Doses** (mg/kg-d)	**0**		**100**		**300**		**1,000**	
	TSH (ng/mL)								
	Male (n = 5−10)								
	Mean (SD)	0.46 (0.42)		3.29 (3.86)		2.65 (2.10)		3.88 (2.98)	
	% of control[a]	--		615%		476%		743%	
	Female (n = 5−10)								
	Mean (SD)	0.46 (0.31)		1.42 (1.11)		3.96 (5.15)		2.43 (1.74)	
	% of control[a]	--		209%		761%		428%	
	T4 (µg/dL)								
	Male (n = 9−10)								
	Mean (SD)	7.87 (1.22)		6.34* (1.22)		6.28* (1.03)		4.97* (0.76)	
	% of control[a]	--		−19%		−20%		−37%	
	Female (n = 9−10)								
	Mean (SD)	5.43 (0.86)		4.96 (0.62)		4.53* (0.88)		4.31* (0.76)	
	% of control[a]	--		−9%		−17%		−21%	
	T3 (ng/dL)								
	Male (n = 9−10)								
	Mean (SD)	64.36 (9.55)		58.78 (13.01)		58.96 (13.17)		64.23 (9.55)	
	% of control[a]	--		−9%		−8%		0%	
	Female (n = 9−10)								
	Mean (SD)	73.4 (14.97)		70.78 (19.18)		67.02 (17.22)		70.31 (16.78)	
	% of control[a]	--		−4%		−9%		−4%	
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage	**Doses** (mg/kg-d)	**0**	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	T4 (nmol/L)								
	Male (n = 4−5)								

Reference and study design	Results							
28-d exposure starting on PNW 11 Thyroid hormones (total T3/T4) were measured by radioimmunoassay	Mean (SD)	40.2 (3.6)	40.4 (5.0)	40.6 (5.3)	49.4 (7.2)	43.3 (1.3)	41.9 (4.6)	35.4 (4.2)	41.4 (3.5)
	% of control[a]	–	0%	1%	23%	8%	4%	−12%	3%
	Female (n = 4–5)**								
	Mean (SD)	41.3 (2.6)	41.9 (3.1)	40.2 (7.3)	37.2 (4.7)	38.6 (1.7)	38 (6.1)	35.8 (5.2)	30.4 (5.9)
	% of control[a]	–	1%	−3%	−10%	−7%	−8%	−13%	−26%
	T3 (nmol/L)								
	Male (n = 4–5)								
	Mean (SD)	0.81 (0.06)	0.84 (0.14)	0.85 (0.16)	0.89 (0.04)	0.97 (0.16)	0.90 (0.13)	0.82 (0.06)	0.89 (0.05)
	% of control[a]	–	4%	5%	10%	20%	11%	1%	10%
	Female (n = 4–5)								
	Mean (SD)	0.91 (0.10)	0.84 (0.15)	0.88 (0.12)	0.81 (0.11)	0.80 (0.09)	0.74 (0.15)	0.92 (0.20)	0.82 (0.13)
	% of control[a]	–	−8%	−3%	−11%	−12%	−19%	1%	−10%
{Saegusa, 2009, 787721@@author-year} Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 Thyroid hormones were measured by electrochemi-luminescence immunoassay in males only	**Doses** (mg/kg-d)[c]	**0**		**15**		**146**		**1,505**	
	TSH (ng/mL)								
	Male, F1, PND 20 (n = 10)								
	Mean (SD)	5.40 (0.62)		6.66 (1.24)		6.07 (1.41)		7.00* (1.31)	
	% of control[a]	–		23%		12%		30%	
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	4.74 (0.62)		5.81 (1.72)		5.36 (1.11)		4.96 (0.8)	
	% of control[a]	–		23%		13%		5%	
	T4 (µg/dL)								
	Male, F1, PND 20 (n = 10)								
	Mean (SD)	4.39 (0.93)		4.20 (0.77)		4.78 (0.49)		4.20 (0.52)	
	% of control[a]	–		−4%		9%		−4%	
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	4.77 (0.7)		4.84 (0.59)		5.21 (0.65)		5.20 (0.98)	
	% of control[a]	–		1%		9%		9%	
	T3 (ng/mL)								
	Male, F1, PND 20 (n = 10)								
	Mean (SD)	1.09 (0.11)		1.13 (0.12)		1.06 (0.08)		0.93* (0.10)	
	% of control[a]	–		4%		−3%		−15%	
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	0.96 (0.06)		0.93 (0.07)		0.88* (0.05)		0.89* (0.06)	
	% of control[a]	–		−3%		−8%		−7%	
Thyroid histopathology									
	Doses (mg/kg-d)								

Reference and study design	Results				
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Male, F0**	**0**	**10**	**101**	**1,008**
	Female, F0	**0**	**14**	**141**	**1,363**
	Male, F1	**0**	**11**	**115**	**1,142**
	Female, F1	**0**	**14**	**138**	**1,363**
	Decreased thyroid follicle size				
	Male, F0 (n = 23–24)				
	Incidence	0/24	0/24	6/24*	20/23*
	Female, F0 (n = 23–24)				
	Incidence	0/24	0/24	5/24*	11/23*
	Male, F1 (n = 22–24)				
	Incidence	0/24	0/24	2/22	11/24*
	Female, F1 (n = 24)				
	Incidence	0/24	1/24	5/24*	13/24*
	Thyroid follicular cell hypertrophy				
	Male, F0 (n = 23–24)				
	Incidence	0/24	0/24	3/24	1/23
	Female, F0 (n = 23–24)				
	Incidence	0/24	0/24	2/24	0/23
	Male, F1 (n = 22–24)				
	Incidence	0/24	0/24	0/22	0/24
	Female, F1 (n = 24)				
	Incidence	0/24	0/24	0/24	0/24
	Thyroid gland histopathology				
	Treatment-related histopathological thyroid changes were not observed in weanling F1 and F2 animals.				
{WIL Research, 2001, 787787@@author-year} Rats, Crl:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown	**Doses** (mg/kg-d)				
	0		**100**	**300**	**1,000**
	Thyroid follicular cell hypertrophy (total incidence, includes all severities)				
	Male (n = 9–10)				
	Incidence	1/10	1/10	5/10	8/9
	Female (n = 9–10)				
	Incidence	0/10	0/10	4/9	7/10
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure in adults starting on PNW 11	**Doses** (mg/kg-d)				
	0	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	Thyroid activation				
	Dose-dependent increases in thyroid activation (i.e., follicle size, epithelial cell height, vacuolization, and nuclear size) were reported qualitatively for both males and females.				
{WIL Research, 1997, 787758@@author-year} Rats, Sprague-Dawley	**Doses** (mg/kg-d)				
	0		**125**	**350**	**1,000**
	Thyroid follicular cell hypertrophy (total incidence, includes all severities)				

Reference and study design	Results			
Gavage 28-d exposure starting on ~PNW 6 followed by a 14-d recovery period	**Male** (n = 6)			
	Incidence	6/6	6/6	6/6	6/6
	Female (n = 6)			
	Incidence	6/6	5/6	6/6	6/6
	Colloid loss (total incidence, includes all severities)			
	Male (n = 6)			
	Incidence	5/6	4/6	6/6	6/6
	Female (n = 6)			
	Incidence	4/6	4/6	6/6	6/6
{Saegusa, 2009, 787721@@author-year} Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11	**Doses** (mg/kg-d)[c]			
		0	**15**	**146**	**1,505**
	Thyroid follicular cell hypertrophy			
	Female, F0 (n = 10)			
	Incidence	3/10	5/10	6/10	9/10*
	Males and females, F1: no treatment-related histopathological effects.			
{Maranghi, 2013, 1927558@@author-year} Mice, BALB/c Females only Diet 28-d exposure starting on PND 26	**Doses** (mg/kg-d)			
		0		**199**	
	Female (n = 6–8)			
	Colloid area (μm^2)			
	Mean (SD)	1,718 (403)		1,270 (452)	
	% of control[a]	–		−26%	
	Follicle area (μm^2)			
	Mean (SD)	2,402 (500)		1,927 (610)	
	% of control[a]	–		−20%	
	Follicle:colloid ratio			
	Mean (SD)	1.41 (0.07)		1.53* (0.07)	
	% of control[a]	–		9%	
Thyroid weight					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal	**Doses** (mg/kg-d)				
	Male, F0	**0**	**10**	**101**	**1,008**
	Female, F0	**0**	**14**	**141**	**1,363**
	Male, F1	**0**	**11**	**115**	**1,142**
	Female, F1	**0**	**14**	**138**	**1,363**
	Relative thyroid weight (mg/100 g BW)				
	Male, F0 (n = 22–24)				
	Mean (SD)	4.28 (0.71)	4.17 (0.77)	4.09 (0.73)	5.17* (1.00)
	% of control[a]	–	−3%	−4%	21%
	Female, F0 (n = 17–24)				
	Mean (SD)	6.38 (0.89)	5.99 (1.27)	6.47 (1.32)	7.20 (1.30)

Reference and study design	Results							
exposure throughout gestation/lactation Thyroid weight measured in adults only	% of control[a]	–	−6%		1%		13%	
	Male, F1 (n = 22–24)							
	Mean (SD)	4.03 (0.79)	4.22 (0.63)		4.15 (0.72)		4.96* (0.87)	
	% of control[a]	–	5%		3%		23%	
	Female, F1 (n = 13–22)							
	Mean (SD)	6.01 (1.01)	6.08 (1.05)		6.54 (1.36)		7.76* (1.36)	
	% of control[a]	–	1%		9%		29%	
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Absolute thyroid weight (mg)								
	Male, F1 (n = 5)								
	Mean (SD)	26 (3)	24 (3)	30 (5)	26 (3)	26 (3)	25 (5)	25 (5)	26 (1)
	% of control[a]	–	−8%	15%	0%	0%	−4%	−4%	0%
	Female, F1 (n = 5)								
	Mean (SD)	24 (5)	21 (3)	19 (4)	20 (5)	22 (4)	20 (4)	19 (6)	22 (3)
	% of control[a]	–	−12%	−21%	−17%	−8%	−17%	−21%	−8%
{WIL Research, 2001, 787787@@author-year} Rats, Crl:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown	**Doses** (mg/kg-d)	**0**		**100**		**300**		**1,000**	
	Relative thyroid weight (mg/100 mg BW)								
	Male (n = 9–10)								
	Mean (SD)	5 (1.2)		5 (1.6)		5 (1.6)		5 (1.3)	
	% of control[a]	–		0%		0%		0%	
	Female (n = 10)								
	Mean (SD)	6 (1.2)		7 (1.8)		6 (1.2)		7 (1.4)	
	% of control[a]	–		17%		0%		17%	
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure starting on PNW 11	**Doses** (mg/kg-d)	**0**	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	Relative thyroid weight (g/g BW × 100,000)								
	Male (n = 3–5)								
	Response	7.33 (1.03)	4.08 (0.36)	6.13 (1.68)	6.97 (0.10)	6.02 (2.09)	6.28 (0.53)	5.54 (0.39)	6.46 (1.14)
	% of control[a]	–	−44%	−16%	−5%	−18%	−14%	−24%	−12%
	Female (n = 4–5)**								
	Response	5.98 (0.60)	6.62 (0.68)	8.98 (1.03)	5.26 (1.35)	7.13 (0.60)	9.52 (0.59)	9.41 (2.26)	9.59 (0.88)
	% of control[a]	–	11%	50%	−12%	19%	59%	57%	60%
{Saegusa, 2009, 787721@@author-year}	**Doses** (mg/kg-d)[c]	**0**		**14.8**		**146.3**		**1,505**	

Reference and study design	Results				
Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	**Relative thyroid weight** (mg/100 g BW)				
	Female, F0 (n = 10)				
	Mean (SD)	5.73 (0.90)	6.75 (0.99)	6.30 (0.80)	7.47* (1.05)
	% of control[a]	–	18%	10%	30%
	Male, F1, PNW 11 (n = 10)				
	Mean (SD)	4.85 (0.69)	5.66 (0.67)	5.78* (0.82)	6.20* (1.03)
	% of control[a]	–	17%	19%	28%
	Female, F1, PNW 11 (n = 10)				
	Mean (SD)	8.20 (2.94)	6.84 (0.81)	7.35 (0.87)	7.72 (0.83)
	% of control[a]	–	−17%	−10%	−6%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[b]Not measured; only control and high-dose values reported for endocrine parameters in the F0 animals.
[c]Time-weighted averages (TWAs) for each exposure group were calculated by multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10−20, PNDs 1−9, and PNDs 9−20 by the number of inclusive days of exposure for each time.

BW = body weight; GD = gestation day; PNW = postnatal week

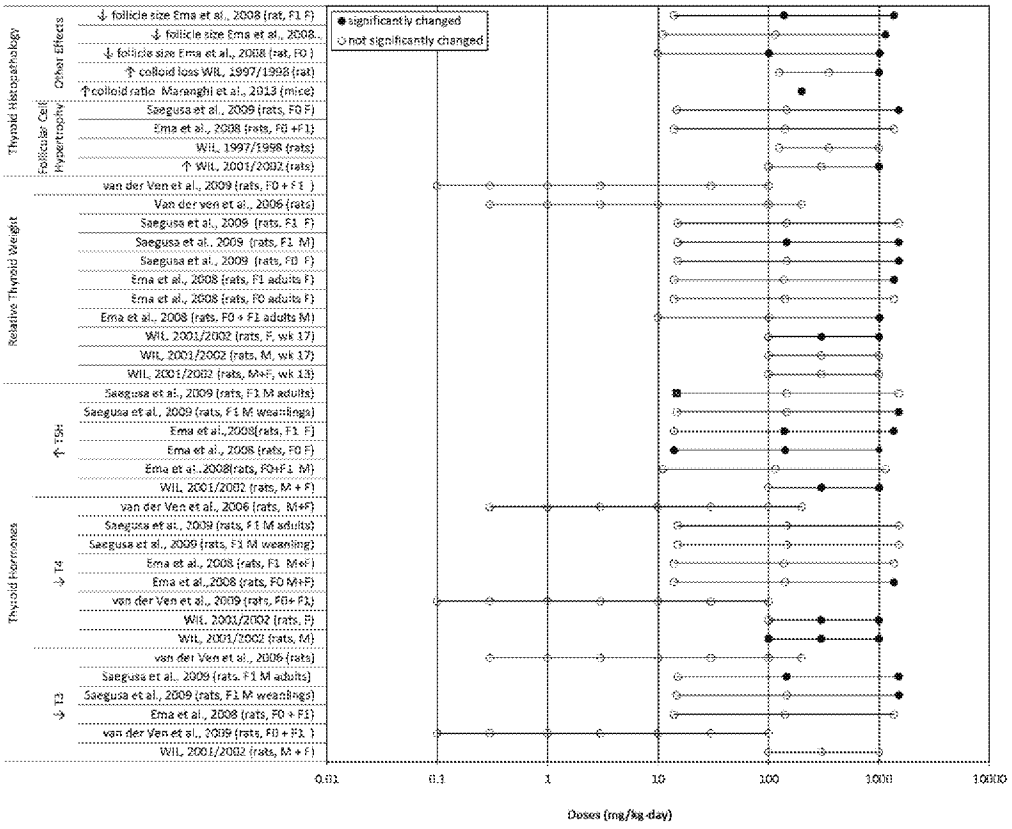


Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Exposure response array of thyroid effects following oral exposure.

1.1.6. Mechanistic Evidence

Available mechanistic data suggest that HBCD may interfere with normal thyroid hormone function. Indirectly, HBCD may decrease circulating thyroid hormone levels by inducing liver xenobiotic enzymes that are responsible for metabolizing thyroid hormones. Directly, HBCD may act via the thyroid receptor and regulate thyroid-responsive genes. Evidence to support these hypothesized modes of action (MOAs) are reviewed below. Other related, but less supported possible mechanisms, such as competition for thyroid hormone binding proteins and dysregulation of deiodinases, are also included in this review. The complex interplay of physiologic processes that regulate thyroid hormone homeostasis and possible sites of disruption by HBCD are summarized in [REF _Ref532802379 \h * MERGEFORMAT] and the text below.

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1.1.6

1.1.6.1 Indirect pathway: Increased clearance of thyroid hormones

Results from short-term in vivo studies suggest that HBCD induces uridine diphosphate glucuronyl transferase (UGT), an enzyme that regulates metabolism and irreversible elimination of T4 {Kelly, 2000, 3045309;Vansell, 2002, 644812;Shelby, 2003, 3045314}. HBCD-mediated activation of UGT has been observed in both rodent and non-mammalian models {Cantón, 2008, 787647;Crump, 2008, 1408111;Crump, 2010, 1403482;Palace, 2008, 1409610;van der Ven, 2006, 787745}. In rats, UGT activity showed dose-related increases in both males and females exposed to up to 200 mg/kg-day {van der Ven, 2006, 787745} and gene transcription in males exposed to 30 and 100 mg/kg-day HBCD {Cantón, 2008, 787647}. Additional support for this mechanism is provided by data obtained from fish and avian models. Activity of liver UGT increased by approximately 45% in juvenile rainbow trout exposed to α- or β-HBCD isomers in the diet for 56 days {Palace, 2008, 1409610}. Similarly, the technical mixture or α-HBCD induced hepatic expression of a UGT1A1 ortholog in chicken embryos {Crump, 2008, 1408111;Crump, 2010, 1403482}. These data suggest that HBCD-mediated induction of UGT could lower serum thyroid hormone levels through increased thyroid hormone catabolism and excretion {Klaassen, 2001, 199716;Kato, 2008, 3045308}. As shown in [REF _Ref532802379 \h * MERGEFORMAT], decreased levels of circulating thyroid hormones trigger activation of HPT axis feedback mechanisms, which stimulate the release of TSH.

Although the exact mechanism by which HBCD induces UGT is unclear, there is some evidence to indicate that this effect may be mediated by interaction with the constitutive androstane receptor (CAR) and/or pregnane X receptor (PXR). Often referred to as xenobiotic sensors, these nuclear receptors bind to numerous exogenous compounds and regulate metabolizing enzymes {Chen, 2003, 3045304;Mackenzie, 2003, 3045310}. HBCD activated CAR in a human breast cancer cell line {Sakai, 2009, 1404688}. Although {Sakai, 2009, 1404688@@author-year} is the only study that directly investigated interaction of HBCD with CAR/PXR, these results are supported by studies in HBCD-exposed animal models showing activation of several other enzymes that are regulated by these nuclear receptors {Omiecinski, 2011, 1610664;Rosenfeld, 2003, 3045311;Ueda, 2002, 3045315}. Upregulation or increased activity of CYP2B1/2 and CYP3A1/3 was reported in HBCD-exposed rats {Germer, 2006, 787665;Cantón, 2008, 787647} and chicken embryos {Crump, 2008, 1408111;Crump, 2010, 1403482}. Pentoxyresorufin-O-depentylase activity, a biomarker of CYP2B1, was also increased in HBCD-exposed

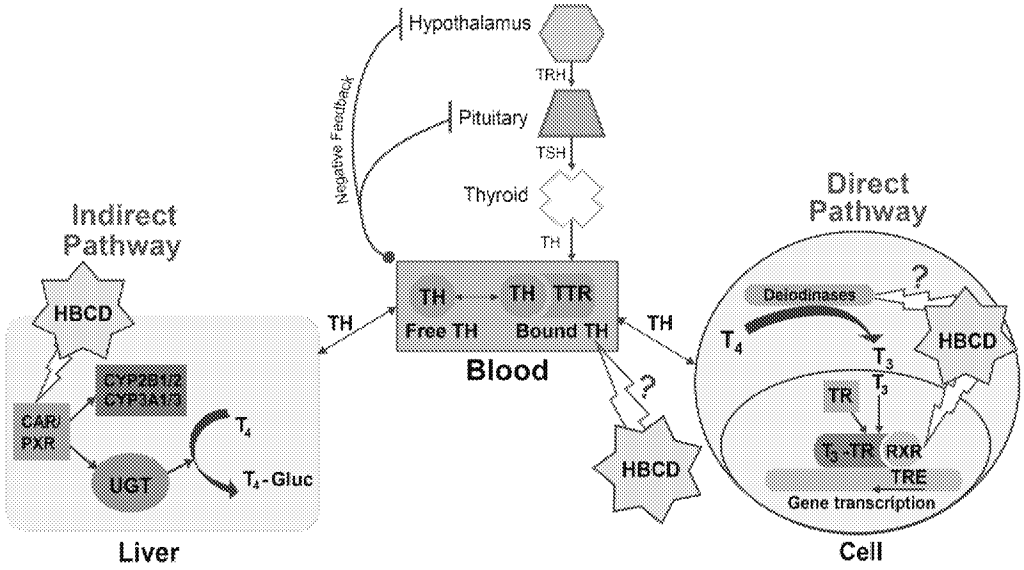
fish {Zhang, 2008, 1927768}. Additionally, liver weight increases in rats and mice are often associated with hepatic microsomal induction {Amacher, 1998, 2912596}; thus, the HBCD-induced liver weight increases (16–108%) observed in rodents {WIL Research, 2001, 787787;Maranghi, 2013, 1927558;Saegusa, 2009, 787721} are consistent with the findings from these mechanistic studies. Taken together, these data support the hypothesis that perturbation of thyroid hormones following HBCD exposure is driven by indirect induction of UGT through interaction with CAR/PXR.

1.1.6.2 Direct pathway: Stimulation of thyroid hormone receptor (TR) signaling at the cellular level

Thyroid hormones bind with the thyroid receptor (TR) to form the thyroid hormone/TR complex. When formed, this complex translocates into the nucleus to activate transcription via the thyroid hormone response element (TRE). Xenobiotic chemicals can alter TRE transcription by interfering with the formation of the thyroid hormone/TR complex or its ability to interact with the TRE {Kitamura, 2005, 1299591}. Although it is unclear whether HBCD binds to the TR, there is evidence to support treatment-related TR activation (e.g., proliferation, gene expression).

Several in vitro models indicate that HBCD may act as a TR agonist. Two studies evaluated the effect of HBCD on rat pituitary tumor cells (GH3 cells) that proliferate via TR activation by T3. Both reported that the technical mixture of HBCD increased GH3 cell proliferation in the presence of T3 {Hamers, 2006, 787675;Schriks, 2006, 787723}. In the absence of T3, α-HBCD, but not other isomers, still induced proliferation; however, the magnitude of the effect was small {Hamers, 2006, 787675}. Maximal proliferation stimulation by HBCD was observed when T3 was added simultaneously, which mimics in vivo conditions.

Interaction of HBCD with the TR was also examined in a *Xenopus laevis* tadpole tail tip regression model that simulates amphibian metamorphosis. In organ culture, the tail tissue responds to T3 by undergoing TR-mediated regression {Furlow, 2004, 3045306;Shaffer, 1963, 3045313}. {Schriks, 2006, 938764@@author-year} demonstrated that the T3-induced tadpole tail tip regression was potentiated by the technical mixture of HBCD. In HeLa cells that constitutively overexpress TRα and were transfected with TRE luciferase construct, HBCD increased TRE transcription by about 1.8-fold {Yamada-Okabe, 2005, 787752}. Two studies using green monkey kidney fibroblast (CV-1) cells transfected with *Xenopus* TR/TRE luciferase constructs provide inconsistent results regarding the effects of HBCD on TR activation {Schriks, 2007, 1927775;Ibhazehiebo, 2011, 1402779}. Notably, this model has less biological relevance in studying TR activation when compared to those that endogenously express the TR (e.g., "T-screen" assay, *X. laevis* tadpole tail tip regression, and HeLa cells).



Indirect Pathway. HBCD induces UGT in the liver, increasing TH elimination, lowering circulating TH levels and activating the hypothalamic-pituitary-thyroid feedback axis. Direct Pathway: HBCD may interfere with TR signaling by interfering with binding to the TRE. Other: HBCD may alter thyroid homeostasis through competitive binding with TTR or dysregulation of deiodinases. CAR/PXR = constituative antrostane receptor/pregnane X receptor; Gluc = glucuronide; RXR = retinoid X receptor; T_4 = Thyroxine; T_3 = triiodothyronine; TH = thyroid hormone; TR = thyroid receptor; TRE = thyroid hormone response element; TRH = thyrotropin-releasing hormone; TSH = thyroid stimulating hormone; TTR = transthretin; UGT = uridine diphosphate glucuronyltransferase;

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Hypothesized MOAs for thyroid effects of HBCD (adapted from {Miller, 2009, 1404905@@author-year})

1.1.6.3 Other mechanistic information

Environmental chemicals can alter circulating levels of free T3 and T4 by competitively binding with the serum transport protein, transthyretin (TTR) {Lans, 1993, 1306578;Schussler, 2000, 3045312} or interacting with deiodinase enzymes {Klammer, 2007, 1297157;Morse, 1993, 784838}. Two in vitro studies provide limited evidence of HBCD interaction with TTR. {Crump, 2008, 1408111@@author-year} reported a >2-fold inhibition of TTR messenger ribonucleic acid (mRNA) transcription in chicken embryonic hepatocytes following exposure to both the technical mixture and α-HBCD for 24 hours, but this effect diminished after treatment for 36 hours. In a TTR replacement assay, α- and β-HBCD showed low potency (IC_{50} > 10 μM), whereas the technical mixture and γ-isomer showed no ability to compete with T4 binding sites {Hamers, 2006, 787675}. Additionally, dysregulation of deiodinase enzymes that catalyze the deiodination of T4 to T3 can disrupt thyroid hormone metabolism {Klammer, 2007, 1297157;Morse, 1993, 784838}. In the liver, total T4 to T3 conversion was decreased by approximately 40% in juvenile rainbow trout fed α-, β-, or γ-isomers for 56 days {Palace, 2008, 1409610}; however, the

same research group later reported that β- and γ-HBCD increased conversion by approximately 60% in the same species after a 32-day dietary exposure {Palace, 2010, 1403364}. Differences in the way enzyme activity was measured in the two experiments may have contributed to the disparate outcomes. Overall, these data provide limited evidence for a role of HBCD in dysregulating the conversion of T4 to T3 in the liver.

1.2. Liver Effects

1.2.1. Human Evidence

The potential for HBCD to affect the liver has not been investigated in humans.

1.2.2. Animal Evidence

Several rodent studies have evaluated hepatic effects, including changes in liver weight, liver chemistry, and histopathology, following oral exposure to HBCD. A summary of liver effects associated with HBCD exposure is presented in [REF _Ref532803903 \h * MERGEFORMAT] and [REF _Ref532803977 \h * MERGEFORMAT]. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. If not otherwise indicated, endpoint measurements were made in adults.

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1.2.1

1.2.2

1.2.2.1 Liver weight

Effects on liver weight were evaluated in eight studies in rats {WIL Research, 2001, 787787;Saegusa, 2009, 787721;Ema, 2008, 787657;WIL Research, 1997, 787758;van der Ven, 2009, 589273;van der Ven, 2006, 787745} and mice {Yanagisawa, 2014, 2343717;Maranghi, 2013, 1927558}. With the exception of three studies that presented only absolute liver weight {Yanagisawa, 2014, 2343717;van der Ven, 2009, 589273;van der Ven, 2006, 787745}, study authors reported both absolute and relative liver weights. This discussion focuses on relative liver weight changes, as this measure has been shown in the general literature to be more informative in evaluating liver toxicity when there are changes in body weight {Bailey, 2004, 782883}; absolute weight data were considered when relative weights were not available.

Statistically significant increases in relative liver weight were reported in five studies in rats {WIL Research, 2001, 787787;Saegusa, 2009, 787721;Ema, 2008, 787657;WIL Research, 1997, 787758}

and mice {Maranghi, 2013, 1927558} that utilized similar dose ranges (10–1,505 mg/kg-day), generally at concentrations ≥100 mg/kg-day.

Study authors reported a significant positive trend with dose for absolute liver weight in adult female, but not male, rats exposed to HBCD for 28 days {van der Ven, 2006, 787745}, but a later study by the same research group did not see a similar effect in F1 rats from a one-generation study {van der Ven, 2009, 589273}. In a study designed to investigate the influence of HBCD exposure on metabolic function {Yanagisawa, 2014, 2343717}, absolute liver weight was examined in male mice dosed once per week for 105 days while being fed either a standard diet or a high-fat diet (created by mixing lard into the feed) at HBCD dose levels (0.002–0.7 mg/kg-week) several orders of magnitude lower than other studies. Changes in absolute liver weight were not observed in mice receiving the standard diet but mice receiving the high-fat diet showed treatment-related increases. The increased absolute liver weight corresponded with significant increases in body weight in these animals.

In three rat studies that evaluated animals 2–8 weeks after the end of exposure, liver weight returned to control levels in all dose groups {WIL Research, 2001, 787787;WIL Research, 1997, 787758;Saegusa, 2009, 787721}.

1.2.2.2 Liver histopathology

Histopathological changes were investigated following oral exposure to HBCD in six studies in rats {WIL Research, 1997, 787758;WIL Research, 2001, 787787;Saegusa, 2009, 787721;Ema, 2008, 787657} and mice {Maranghi, 2013, 1927558;Yanagisawa, 2014, 2343717}. Increased hepatocellular vacuolation, which can reflect a normal physiological process as well as a response to a toxic agent {Henics, 1999, 783473}, was the most consistently observed histopathological change, with effects seen in male and female rats and female mice following multiple exposure durations at doses ranging from 100 to 1,505 mg/kg-day {WIL Research, 2001, 787787;Maranghi, 2013, 1927558;Saegusa, 2009, 787721;WIL Research, 1997, 787758}. One of these studies stained liver sections with lipid- and glycogen-specific stains (Oil Red O and periodic acid Schiff's reagent, respectively) and characterized the vacuoles as lipid filled {WIL Research, 2001, 787787}. With the exception of hypertrophy, which was increased in high-dose females in the study by {WIL Research, 2001, 787787@@author-year}, no other significant histopathological changes were reported in the available rat studies; however, some histopathologic changes were observed in mouse studies. Low HBCD exposures (up to 0.7 mg/kg-week) in male mice showed no histological changes in mice fed a standard diet; however, increases in microvesicular fatty changes (steatosis) and hypertrophy (characterized as hepatocyte ballooning) were observed in the high-dose group given a high-fat diet relative to the high-fat controls. Confidence in these findings is reduced because other dose groups were not evaluated histologically and data were presented qualitatively only {Yanagisawa, 2014, 2343717}. In a second mouse study, statistically significant increases in the incidence of lymphocytic infiltration and tissue congestion, indicators of inflammation, were observed in female mice administered 199 mg/kg-day {Maranghi, 2013, 1927558}.

In two rat studies that evaluated animals 2–4 weeks after the end of exposure, histopathological changes returned to control levels in all dose groups {WIL Research, 2001, 787787;WIL Research, 1997, 787758}.

1.2.2.3 Liver chemistry

Changes in serum liver enzyme levels were investigated as potential indicators of liver damage following short-term and subchronic oral exposure to HBCD in five studies in rats {WIL Research, 1997, 787758;WIL Research, 2001, 787787;van der Ven, 2009, 589273;van der Ven, 2006, 787745} and mice {Yanagisawa, 2014, 2343717}.

Measures of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicators of hepatocellular injury, showed no biologically or statistically significant increases with HBCD exposure; indeed, animals in the high-dose groups often showed decreases in these enzyme levels {Yanagisawa, 2014, 2343717;WIL Research, 1997, 787758;WIL Research, 2001, 787787;van der Ven, 2009, 589273;van der Ven, 2006, 787745}. Although it is generally accepted that increases in serum ALT greater than 100% of controls is suggestive of hepatocellular damage {EMEA, 2008, 3056793;Boone, 2005, 782862}, the biological significance of decreased aminotransferase levels is unclear.

Serum γ-glutamyltransferase (GGT) and serum alkaline phosphatase (ALP) activities, markers of hepatobiliary injury, were also reported in four studies {WIL Research, 1997, 787758;WIL Research, 2001, 787787;van der Ven, 2009, 589273;van der Ven, 2006, 787745}. GGT was significantly increased in male and female rats exposed to 1,000 mg/kg-day for 90 days; this effect was not observed following a 4-week recovery period {WIL Research, 2001, 787787} or a shorter (28-day) exposure {WIL Research, 1997, 787758}. In general, ALP activity was consistently decreased, sometimes statistically significantly, in male and female rats {van der Ven, 2009, 589273;van der Ven, 2006, 787745;WIL Research, 2001, 787787;WIL Research, 1997, 787758}. Although decreased ALP levels are not generally associated with liver injury, they can be a marker of vitamin B_6 (pyridoxal phosphate) or zinc deficiency {Hall, 2012, 2718645;Waner, 1991, 2850005}.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Evidence pertaining to liver effects in animals following exposure to HBCD

Reference and study design	Results				
Liver weight					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal	**Doses** (mg/kg-d)				
	Male, F0	**0**	**10**	**101**	**1,008**
	Female, F0	**0**	**14**	**141**	**1,363**
	F1 offspring[a]	**0**	**17**	**168**	**1,570**
	Male, F1	**0**	**11**	**115**	**1,142**
	Female, F1	**0**	**14**	**138**	**1,363**
	F2 offspring[a]	**0**	**15**	**139**	**1,360**
	Relative liver weight (g/100 g BW)				
	Male, F0 (n = 22–24)				
	Mean (SD)	3.23 (0.26)	3.33 (0.24)	3.41* (0.31)	4.06* (0.22)
	% of control[b]	–	3%	6%	26%
	Female, F0 (n = 17–24)				

Reference and study design	Results							
exposure throughout gestation/lactation	Mean (SD)	4.69 (0.52)		4.76 (0.65)	4.88 (0.48)		6.07* (0.47)	
	% of control[b]	–		1%	4%		29%	
	Male, F1, PND 26 (n = 17–23)							
	Mean (SD)	4.60 (0.37)		4.60 (0.32)	5.05* (0.32)		6.00* (0.44)	
	% of control[b]	–		0%	10%		30%	
	Female, F1, PND 26 (n = 14–23)							
	Mean (SD)	4.57 (0.35)		4.59 (0.28)	5.02* (0.32)		6.07* (0.36)	
	% of control[b]	–		0%	10%		33%	
	Male, F1, adult (n = 22–24)							
	Mean (SD)	3.27 (0.18)		3.34 (0.26)	3.37 (0.25)		3.86* (0.28)	
	% of control[b]	–		2%	3%		18%	
	Female, F1, adult (n = 13–22)							
	Mean (SD)	4.18 (0.42)		4.39 (0.44)	4.38 (0.47)		5.05* (0.50)	
	% of control[b]	–		5%	5%		21%	
	Male, F2, PND 26 (n = 13–22)							
	Mean (SD)	4.72 (0.59)		4.74 (0.35)	5.04* (0.4)		6.00* (0.25)	
	% of control[b]	–		0%	7%		27%	
	Female, F2, PND 26 (n = 13–22)							
	Mean (SD)	4.70 (0.27)		4.70 (0.28)	4.94 (0.32)		5.89* (0.44)	
	% of control[b]	–		0%	5%		25%	
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Absolute liver weight (g)								
	Male, F1, PNW 11 (n = 4–5)								
	Mean (SD)	11.9 (1.5)	12.3 (0.4)	12.7 (0.8)	14.4 (2.0)	12.2 (1.7)	12.1 (0.8)	14.0 (2.8)	12.0 (0.5)
	% of control[b]	–	3%	7%	21%	3%	2%	18%	1%
	Female, F1, PNW 11 (n = 4–5)								
	Mean (SD)	7.7 (0.9)	7.9 (0.8)	7.8 (1.4)	8.3 (0.5)	7.7 (0.8)	8.3 (0.5)	9.0 (1.1)	8.4 (0.6)
	% of control[b]	–	3%	1%	8%	0%	8%	17%	9%
{WIL Research, 2001, 787787@@author-year} Rats, Crl:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period	**Doses** (mg/kg-d)	**0**		**100**	**300**		**1,000**	
	Relative liver weight (g/100 g BW)							
	Male (n = 10)							
	Mean (SD)	2.71 (0.12)		3.18* (0.23)	3.13* (0.27)		3.86* (0.16)	
	% of control[b]	–		17%	17%		42%	
	Female (n = 10)							
	Mean (SD)	2.89 (0.21)		3.58* (0.27)	3.58* (0.35)		4.31* (0.29)	

Reference and study design	Results							
Recovery data not shown	% of control[b]	–	24%		24%		49%	
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure starting on PNW 11	**Doses** (mg/kg-d)	**0**	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	Absolute liver weight (g)								
	Male (n = 4–5)								
	Mean (SD)	13.9 (0.7)	17.1 (3.4)	16.2 (3.0)	15.0 (1.6)	17.7 (2.3)	15.7 (0.5)	16.4 (2.3)	16.4 (3.2)
	% of control[b]	–	23%	17%	8%	27%	13%	18%	18%
	Female (n = 4–5)**								
	Mean (SD)	9.7 (1.0)	8.9 (1.1)	8.6 (1.3)	9.5 (0.4)	8.9 (0.6)	11.0 (1.0)	13.0 (0.5)	11.6 (0.6)
	% of control[b]	–	−8%	−11%	−2%	−8%	13%	34%	20%
{WIL Research, 1997, 787758@@author-year} Rats, Sprague-Dawley Gavage 28-d exposure starting on ~PNW 6 followed by a 14-d recovery period Recovery data not shown	**Doses** (mg/kg-d)	**0**		**125**		**350**		**1,000**	
	Relative liver weight (g/100 g BW)								
	Male (n = 6)								
	Mean (SD)	3.68 (0.16)		4.05 (0.24)		4.29* (0.29)		4.76* (0.44)	
	% of control[b]	–		10%		17%		29%	
	Female (n = 6)								
	Mean (SD)	3.84 (0.39)		4.47* (0.26)		4.69* (0.59)		5.30* (0.25)	
	% of control[b]	–		16%		22%		38%	
{Saegusa, 2009, 787721@@author-year} Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	**Doses** (mg/kg-d)[c]	**0**		**15**		**146**		**1,505**	
	Relative liver weight (g/100 g BW)								
	Male, F1, PND 20 (n = 10)								
	Mean (SD)	3.68 (0.11)		3.82 (0.31)		3.98 (0.15)		4.66* (0.35)	
	% of control[b]	–		4%		8%		27%	
	Female, F1, PND 20 (n = 10)								
	Mean (SD)	3.77 (0.17)		3.83 (0.23)		4.01 (0.25)		4.83* (0.26)	
	% of control[b]	–		2%		6%		28%	
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	3.45 (0.27)		3.81* (0.23)		3.58 (0.24)		3.53 (0.22)	
	% of control[b]	–		10%		4%		2%	
	Female, F1, PNW 11 (n = 10)								
	Mean (SD)	3.35 (0.20)		3.59 (0.19)		3.44 (0.25)		3.30 (0.22)	
	% of control[b]	–		7%		3%		−1%	
	Doses (mg/kg-wk)								

Reference and study design	Results			
{Yanagisawa, 2014, 2343717@@author-year} Mice, C57BL/6 Males only Gavage Animals dosed once weekly 15-week exposure starting on PNW 6 Dose groups split between standard and high-fat diets		**0**	**0.00175**	**0.035**	**0.7**
	Absolute liver weight (mg), standard diet				
	Male (n = 6)				
	Mean (SE)	1,261 (54.8)	1,283 (36.8)	1,159 (21.9)	1,165 (49.4)
	% of control[b]	–	2%	−8%	−8%
	Absolute liver weight (mg), high-fat diet				
	Male (n = 6)				
	Mean (SE)	1,405 (96.4)	1,622 (164)	1,662* (87.9)	1,790* (153)
	% of control[b]	–	15%	18%	27%
{Maranghi, 2013, 1927558@@author-year} Mice, BALB/c Females only Diet 28-d exposure starting on PND 26	**Doses** (mg/kg-d)	**0**		**199**	
	Relative liver weight (%)				
	Female (n = 10–15)				
	Mean (SD)	4.38 (0.49)		5.67* (0.4)	
	% of control[b]	–		29%	
Liver histopathology					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2: continuous maternal exposure throughout gestation/ lactation	**Doses** (mg/kg-d)				
	Male, F0	**0**	**10**	**101**	**1,008**
	Female, F0	**0**	**14**	**141**	**1,363**
	F1 offspring[a]	**0**	**17**	**168**	**1,570**
	Male, F1	**0**	**11**	**115**	**1,142**
	Female, F1	**0**	**14**	**138**	**1,363**
	F2 offspring[a]	**0**	**15**	**139**	**1,360**
	Histopathological findings				
	Histopathological evaluation did not observe any significant effects with HBCD exposure.				
{WIL Research, 2001, 787787@@author-year} Rats, Crl:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown	**Doses** (mg/kg-d)	**0**	**100**	**300**	**1,000**
	Hepatocellular hypertrophy				
	Male (n = 10)				
	Incidence	0/10	0/10	0/10	0/10
	Female (n = 10)				
	Incidence	0/10	0/10	0/10	5/10
	Hepatocellular vacuolation				
	Male (n = 9–10)				
	Incidence	2/10	6/10	5/10	6/9
	Female (n = 10)				

Reference and study design	Results			
	Incidence	3/10	6/10	5/10	9/10
	Other histopathological findings				
	Inflammation was also observed in animals from every treatment group with no pattern related to dose.				
{WIL Research, 1997, 787758@@author-year} Rats, Sprague-Dawley Gavage 28-d exposure starting on ~PNW 6 followed by a 14-d recovery period Recovery data not shown	**Doses** (mg/kg-d)				
		0	**125**	**350**	**1,000**
	Hepatocellular vacuolation				
	Male (n = 6)				
	Incidence	0/6	0/6	0/6	0/6
	Female (n = 6)				
	Incidence	1/6	4/6	2/6	5/6
	Other histopathological findings				
	Inflammation was also observed in animals from every treatment group with no pattern related to dose.				
{Saegusa, 2009, 787721@@author-year} Crj:CD(SD)IGS, rat Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	**Doses** (mg/kg-d)[c]				
		0	**15**	**146**	**1,505**
	Hepatocellular vacuolar degeneration				
	Male, F1, PND 20 (n = 10)				
	Incidence	0/10	0/10	0/10	6/10*
	Female, F1, PND 20 (n = 10)				
	Incidence	0/10	0/10	0/10	6/10*
{Yanagisawa, 2014, 2343717@@author-year} Mice, C57BL/6 Males only Gavage Animals dosed once weekly 15-wk exposure starting on PNW 6 Dose groups split between standard and high-fat diets	**Doses** (mg/kg-wk)				
		0	**0.00175**	**0.035**	**0.7**
	Hepatocyte ballooning				
	The study authors observed development of hepatocyte ballooning following oral high-dose exposure in male mice fed a high-fat diet.				
	Microvesicular fatty changes				
	The study authors observed development of severe microvesicular fatty changes following oral high-dose exposure in male mice fed a high-fat diet.				
	Treatment-related effects were not observed in mice fed a standard diet.				
{Maranghi, 2013, 1927558@@author-year} BALB/c, mice Females only Diet 28-d exposure starting on PND 26	**Doses** (mg/kg-d)				
			0		**199**
	Periportal lymphatic filtration				
	Incidence		0/10		6/8*
	Tissue congestion				
	Incidence		0/10		6/8*
	Vacuolation in hepatocytes				
	Incidence		0/10		5/8*

Reference and study design	Results							
Liver chemistry								
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	ALT (U/L)								
	Male (n = 4–5)								
	Mean (SD)	37.3 (1.8)	33.6 (4.7)	43.6 (7.8)	43.1 (4.2)	43.3 (4.4)	40.3 (6.8)	38.2 (4.7)	37.2 (2.6)
	% of control[b]	–	−10%	17%	16%	16%	8%	2.4%	0%
	Female (n = 5)								
	Mean (SD)	34.7 (3.3)	37.5 (6.5)	39.7 (12.6)	37.3 (4.8)	33.5 (6.2)	30.7 (6.2)	33.9 (10.4)	34.0 (4.6)
	% of control[b]	–	8%	14%	7%	−3%	−12%	−2%	−2%
	ALP (U/L)								
	Male (n = 4–5)								
	Mean (SD)	3.22 (2.24)	4.40 (2.31)	3.28 (1.76)	4.80 (2.79)	3.38 (1.90)	3.20 (0.85)	4.60 (2.43)	3.76 (1.90)
	% of control[b]	–	37%	2%	49%	5%	−1%	43%	17%
	Female (n = 5)**								
	Mean (SD)	3.78 (1.97)	2.70 (2.37)	3.82 (3.23)	2.64 (0.95)	1.14 (0.53)	3.82 (1.64)	2.66 (1.55)	1.28 (0.59)
	% of control[b]	–	−29%	1%	−30%	−70%	1%	−30%	−66%
{WIL Research, 2001, 787787@@author-year} Rats, Crl:CD(SD)IGS BR Gavage 90-d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown	**Doses** (mg/kg-d)	**0**		**100**	**300**		**1,000**		
	ALT (U/L)								
	Male (n = 9–10)								
	Mean (SD)	40 (12.8)		31 (4.8)	40 (12)		33 (6)		
	% of control[b]	–		−22%	0%		−18%		
	Female (n = 10)								
	Mean (SD)	28 (4.9)		30 (5.5)	31 (11.7)		35 (10.2)		
	% of control[b]	–		7%	11%		25%		
	ALP (U/L)								
	Male (n = 10)								
	Mean (SD)	103 (21.5)		87 (11.3)	97 (20.1)		87 (17.6)		
	% of control[b]	–		−16%	−6%		−16%		
	Female (n = 10)								
	Mean (SD)	58 (19.4)		38* (10.7)	39* (10.7)		34* (11.1)		
	% of control[b]	–		−34%	−33%		−41%		
	AST (U/L)								
	Male (n = 9–10)								

Reference and study design	Results							
	Mean (SD)	89 (21.9)	74 (16.4)	75 (16.9)	67 (10.9)			
	% of control[b]	–	−17%	−16%	−25%			
	Female (n = 10)							
	Mean (SD)	83 (17.6)	86 (25.5)	72 (19.1)	77 (30.8)			
	% of control[b]	–	4%	−13%	−7%			
	GGT (U/L)							
	Male (n = 9−10)							
	Mean (SD)	0 (0)	0 (0.4)	0 (0.7)	1* (1.2)			
	% of control[b]	n/a	n/a	n/a	n/a			
	Female (n = 10)							
	Mean (SD)	0 (0)	0 (0.4)	0 (0.7)	2* (1.7)			
	% of control[b]	n/a	n/a	n/a	n/a			
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure starting on PNW 11	**Doses** (mg/kg-d)	**0**	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	ALT (U/L)								
	Male (n = 3−5)								
	Mean (SD)	44.5 (5.9)	40.9 (4.1)	44.3 (10.3)	38.2 (3.6)	45.0 (14.3)	42.7 (11.0)	40.6 (8.1)	39.2 (10.9)
	% of control[b]	–	−8%	0%	−14%	1%	−4%	−9%	−12%
	Female (n = 3−5)								
	Mean (SD)	43.4 (4.6)	44.7 (6.5)	39.8 (4.5)	40.5 (6.7)	34.6 (6.6)	38.2 (5.0)	36.0 (5.2)	42.5 (7.5)
	% of control[b]	–	3%	−8%	−7%	−20%	−12%	−17%	−2%
	ALP (U/L)								
	Male (n = 3−5)								
	Mean (SD)	7.34 (5.59)	5.30 (3.66)	3.68 (1.82)	7.43 (7.43)	4.88 (5.75)	5.10 (2.54)	2.74 (1.61)	3.48 (1.95)
	% of control[b]	–	−28%	−50%	1%	−34%	−31%	−63%	−53%
	Female (n = 3−5)**								
	Mean (SD)	4.66 (2.91)	3.10 (2.76)	4.74 (2.50)	3.72 (2.14)	2.30 (1.21)	2.36 (0.33)	2.73 (1.55)	2.42 (2.71)
	% of control[b]	–	−33%	2%	−20%	−51%	−49%	−41%	−48%
{WIL Research, 1997, 787758@@author-year} Rats, Sprague-Dawley Gavage 28-d exposure starting on ~PNW 6 followed by a 14-d recovery period	**Doses** (mg/kg-d)	**0**	**125**	**350**	**1,000**				
	ALT (U/L)								
	Male (n = 6)								
	Mean (SD)	31 (4.9)	23* (5.4)	21* (2.3)	23* (3.5)				
	% of control[b]	–	−26%	−32%	−26%				
	Female (n = 6)								

Reference and study design	Results				
Recovery data not shown	Mean (SD)	26 (2.1)	24 (3.7)	27 (3.5)	26 (7.9)
	% of control[b]	–	−8%	4%	0%
	ALP (U/L)				
	Male (n = 6)				
	Mean (SD)	199 (40.9)	149 (24.7)	165 (34.6)	154 (37.1)
	% of control[b]	–	−25%	−17%	−23%
	Female (n = 6)				
	Mean (SD)	100 (29.7)	87 (11.8)	85 (20.4)	74 (9.7)
	% of control[b]	–	−13%	−15%	−26%
	AST (U/L)				
	Male (n = 6)				
	Mean (SD)	80 (18.3)	63* (5.9)	65 (5.4)	61* (6.8)
	% of control[b]	–	−21%	−19%	−24%
	Female (n = 6)				
	Mean (SD)	75 (13.0)	63 (11.5)	61 (9.6)	62 (9.9)
	% of control[b]	–	−16%	−19%	−17%
	GGT (U/L)				
	Male (n = 6)				
	Mean (SD)	1 (0.4)	1 (0.5)	1 (0.5)	1 (0.4)
	% of control[b]	–	0%	0%	0%
	Female (n = 6)				
	Mean (SD)	1 (0.8)	1 (0.8)	1 (0.9)	1 (0.4)
	% of control[b]	–	0%	0%	0%
{Yanagisawa, 2014, 2343717@@author-year} Mice, C57BL/6 Males only Gavage Animals dosed once weekly 15-week exposure starting on PNW 6 Dose groups split between standard and high-fat diets	**Doses** (µg/kg BW)	**0**	**1.75**	**35**	**700**
	ALT (IU/L), standard diet				
	Male (n = 5−6)				
	Mean (SE)	13.6 (1.04)	15.0 (1.18)	14.2 (1.59)	10.5 (0.22)
	% of control[b]	–	10%	4%	−23%
	ALT (IU/L), high-fat diet				
	Male (n = 5−6)				
	Mean (SE)	34.5 (8.43)	43.0 (15.0)	60.0 (12.2)	61.5 (10.2)
	% of control[b]	–	25%	74%	78%
	AST (IU/L), standard diet				
	Male (n = 5−6)				
	Mean (SE)	73.0 (8.86)	74.2 (7.59)	66.6 (6.57)	46.0* (7.96)
	% of control[b]	–	2%	−9%	−37%
	AST (IU/L), high-fat diet				
	Male (n = 5−6)				
	Mean (SE)	79.7 (7.44)	78.7 (8.58)	101 (8.39)	85.2 (7.50)
	% of control[b]	–	−1%	27%	7%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]F1 and F2 offspring presented as mean maternal gestational and lactational F0 and F1 doses, respectively.
[b]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[c]TWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.

SE = standard error

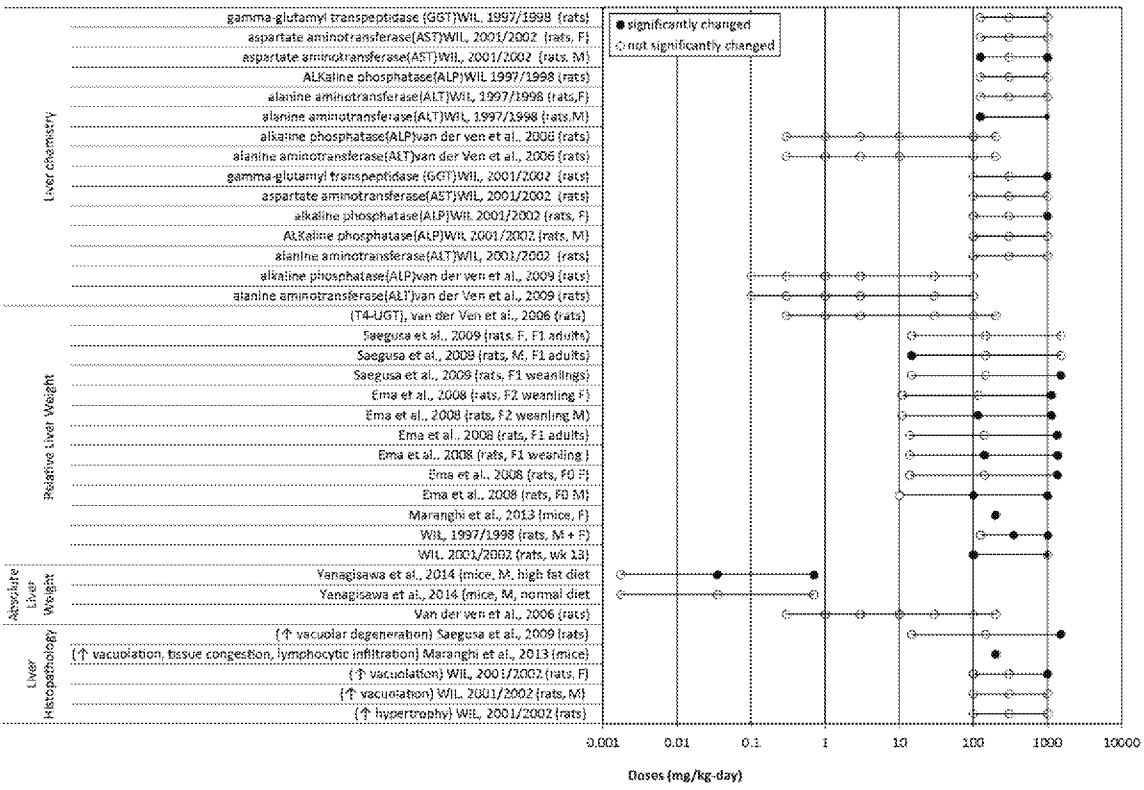


Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Exposure response array of liver effects following oral exposure.

1.2.3. Mechanistic Evidence

Studies have reported a generally consistent pattern of increased liver weight related to HBCD exposure. Increased liver weight is often correlated with induction of hepatic microsomal enzymes, although the level of induction does not necessarily reflect the magnitude of weight change, nor it is a requirement for liver weight increases {Amacher, 1998, 2912596}. HBCD has been shown to induce the expression of several hepatic microsomal enzymes {Germer, 2006, 787665;Crump, 2008, 1408111;Crump, 2010, 1403482}. Specifically, dose-related increases in liver CYP3A1 and CYP2B1 protein levels were observed in rats exposed to HBCD via diet {Germer, 2006, 787665}. In addition, dose-related increases in CYP2H1 and CYP3A37 mRNA levels were observed in chicken hepatocytes following in ovo {Crump, 2010, 1403482} and in vitro exposure {Crump, 2008, 1408111}. Furthermore, some data suggest that induction of hepatic microsomal enzymes responsible for conjugation and

elimination of thyroid hormones may contribute to HBCD-mediated effects related to thyroid perturbation (Section 1.2.1, Mechanistic Evidence). Liver weight changes are also associated with increased hepatocellular hypertrophy and hyperplasia. Hypertrophy was reported in high-dose animals in two studies {WIL Research, 2001, 787787;Yanagisawa, 2014, 2343717}; however, hyperplasia was not noted.

HBCD may also impair lipid homeostasis. Several studies observed increased vacuolation in hepatocytes {WIL Research, 2001, 787787;Maranghi, 2013, 1927558;Saegusa, 2009, 787721;WIL Research, 1997, 787758}. The only study to evaluate vacuole contents indicated that they predominantly consisted of lipid {WIL Research, 2001, 787787}. Chemically-induced impairment of fatty acid metabolism in cells with high energy demands, such as hepatocytes, has been shown to promote accumulation of triglycerides, which form nonmembrane bound vacuoles in cells (i.e., fatty change) {Wheater, 1996, 3449178}. Various gene expression studies lend supportive evidence for HBCD-mediated disruption of genes involved in lipid metabolism and transport. A 28-day study in rats reported inhibition of peroxisome proliferator-activated receptor (PPAR)-mediated genes involved in lipid metabolism, particularly in females {Cantón, 2008, 787647}. Statistically significant increases in liver triglyceride levels as well as PPAR-mediated genes involved in lipid metabolism (PPARg) and transport (FSp27) were also observed in mice exposed to 0.7 mg/kg-week HBCD while being fed a high-fat diet {Yanagisawa, 2014, 2343717}.

HBCD-mediated alterations in the regulation of lipid metabolism have also been observed in avian species and in vitro. HBCD decreased the mRNA expression of liver fatty acid binding protein in chicken hepatocytes in vitro and following in ovo exposure {Crump, 2010, 1403482;Crump, 2008, 1408111}. The observed effects on lipid homeostasis may be a direct effect or secondary to perturbation of thyroid function. In humans and animal models, hypothyroidism is thought to be associated with altered liver metabolism and increased triglycerides and cholesterol, as well as non-alcoholic fatty liver disease {Eshraghian, 2014, 3063058;Pucci, 2000, 3063072}. HBCD studies that evaluated serum lipid profiles did not report any significant changes in serum cholesterol or triglyceride levels in exposed rats {WIL Research, 2001, 787787;van der Ven, 2006, 787745} or mice {Yanagisawa, 2014, 2343717} fed a standard diet; however, statistically significant increases in levels of liver triglycerides were reported in mice exposed concurrently to HBCD and a high-fat diet {Yanagisawa, 2014, 2343717}.

The lack of increased incidence of necrosis or apoptosis and/or serum enzymatic markers of hepatocellular damage suggests that HBCD is not highly cytotoxic. However, there is evidence to suggest the exposure to HBCD can increase the production of reactive oxygen species (ROS). Dose-related increases in ROS were observed in human hepatocyte and carcinoma cell lines following in vitro exposures {An, 2013, 1927550;Hu, 2009, 837636}.

1.3. Reproductive Effects

1.2.3

1.2.4

1.3.1. Female Reproductive Effects

1.3

1.3.1

1.3.1.1 Human evidence

The potential for HBCD to affect the female reproductive system has not been investigated in humans.

1.3.1.2 Animal evidence

Evidence to inform the potential for HBCD to induce female reproductive effects comes from five studies in rats {Ema, 2008, 787657;Saegusa, 2009, 787721;van der Ven, 2009, 589273;WIL Research, 2001, 787787; WIL Research, 1997, 787758} and one study in mice {Maranghi, 2013, 1927558} with exposure durations ranging from 28 days to two generations. Endpoints evaluated in these studies include fertility and pregnancy outcomes, hormone levels, markers of reproductive differentiation and development, and reproductive organ weights. Evidence pertaining to female reproductive effects in experimental animals following oral exposure to HBCD is summarized in [REF _Ref532817487 \h * MERGEFORMAT] and [REF _Ref532804173 \h * MERGEFORMAT]. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. If not otherwise indicated, endpoint measurements were made in adults.

Fertility and pregnancy outcomes were evaluated in three rat studies {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721}. Dose-related decreases in pregnancy incidence in the F0 and F1 dams was reported in the two-generation reproductive toxicity study using doses up to approximately 1,300 mg/kg-day HBCD {Ema, 2008, 787657}. In the F1 females, a 36–37% decrease in the number of primordial follicles was reported at approximately 140 mg/kg-day HBCD or greater received throughout gestation, lactation, and adulthood ($p<0.05$) {Ema, 2008, 787657}. This endpoint was only evaluated in the F1 females. The one-generation reproductive toxicity study, using doses up to 100 mg/kg-day HBCD, reported no significant trend in successful matings, defined as the rate of matings resulting in offspring {van der Ven, 2009, 589273}. The results from {van der Ven, 2009, 589273@@author-year} are not directly comparable to the findings of {Ema, 2008, 787657@@author-year} due to the low doses used by investigators (i.e., a dose range lower than doses associated with effects in {Ema, 2008, 787657@@author-year}). Incidence of pregnancy was not measured in the developmental study using doses up to approximately 1,500 mg/kg-day HBCD because the study began with previously impregnated females {Saegusa, 2009, 787721}. Other measures of fertility and pregnancy outcomes (e.g., gestational duration, number of implantation sites, litter size) reported in these

three studies showed no effect with HBCD exposure studies {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721}.

HBCD-induced changes in reproductive hormone concentrations were examined in both rats {Ema, 2008, 787657} and mice {Maranghi, 2013, 1927558}. {Ema, 2008, 787657@@author-year} observed elevated follicle-stimulating hormone (FSH) concentrations (41%) only in F0 rats exposed to approximately 1,300 mg/kg-day; serum levels of estradiol, testosterone, progesterone, and luteinizing hormone (LH) were not affected. Statistically significant increases in serum testosterone levels (57%) were reported in female mice exposed to 199 mg/kg-day for 28 days {Maranghi, 2013, 1927558}, resulting in a 56% elevation in the testosterone/17β-estradiol ratio.

Effects on reproductive differentiation and development were evaluated in three studies in rats {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721}. Although {van der Ven, 2009, 589273@@author-year} reported a dose-related delay in vaginal opening, a measurement of puberty onset, at concentrations up to 100 mg/kg-day, no treatment-related effects were observed in the other two studies that used concentrations up to 1,505 mg/kg-day {Ema, 2008, 787657;Saegusa, 2009, 787721}. There were no HBCD-mediated effects on anogenital distance (AGD) {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721}.

Treatment-related effects on female reproductive organ weights were evaluated in six studies using both rats {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721;WIL Research, 2001, 787787;WIL Research, 1997, 787758} and mice {Maranghi, 2013, 1927558}. Absolute uterine weights were decreased by 17–23% in a 90-day oral study in rats {WIL Research, 2001, 787787}, but the decreases were not dose-related and returned to control levels after a 4-week recovery period. Absolute, but not relative, uterine weight showed a statistically significant decrease (22%) in F2 rats (PND 26) in the high-dose group (approximately 1,300 mg/kg-day) {Ema, 2008, 787657}; no exposure-related effects on uterine weight were observed in F1 animals. No other clear treatment-related effects were observed on absolute or relative uterine {van der Ven, 2009, 589273;Saegusa, 2009, 787721;Maranghi, 2013, 1927558} or ovary weights {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721;WIL Research, 2001, 787787;WIL Research, 1997, 787758}.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Evidence pertaining to female reproductive effects in animals following exposure to HBCD

Reference and study design	Results				
Fertility and pregnancy outcomes					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation	**Doses** (mg/kg-d)				
	Female, F0	**0**	**14**	**141**	**1,363**
	Female, F1	**0**	**14**	**138**	**1,363**
	Incidence of pregnant females				
	Female, F0 (n = 23–24)				
	Incidence	24/24	22/24	20/24	19/23
	Female, F1 (n = 21–24)				

Reference and study design	Results							
F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	Incidence	23/24		23/24		21/24		21/24
	Primordial follicles (count)							
	Female, F1 (n = 10)							
	Mean (SD)	316.3 (119.5)		294.2 (66.3)		197.9* (76.9)		203.4* (79.5)
	% of control[a]	–		−7%		−37%		−36%
	Other pregnancy outcomes							
	No dose-related changes in other outcomes (e.g., number of implantation sites, gestation duration, litter size) reported in either generation							
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)							
	0	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Successful matings							
	Female, F0 (n = 8−10)							
	Incidence	8/10	8/10	4/10	7/10	8/10	6/8	6/10	6/10
	Other pregnancy outcomes							
	No significant dose-response trend in other outcomes (e.g., number of implantation sites, gestation duration, litter size)							
{Saegusa, 2009, 787721@@author-year} Crj:CD(SD)IGS, rat Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	**Doses** (mg/kg-d)[c]							
	0		**15**		**146**		**1,505**	
	Pregnancy outcomes							
	No dose-related effect on pregnancy outcomes (e.g., number of implantation sites, gestation duration, litter size)							
Hormonal measures								
	Doses (mg/kg-d)							
	Female, F0	**0**		**14**		**141**		**1,363**
	Female, F1	**0**		**14**		**138**		**1,363**
	FSH (ng/mL)							
	Female, F0 (n = 8)							
	Mean (SD)	4.17 (0.51)		4.84 (0.63)		4.88 (1.05)		5.86* (1.11)
	% of control[a]	–		16%		17%		41%
	Female, F1 (n = 8)							
	Mean (SD)	5.89 (1.60)		6.07 (0.60)		6.33 (0.82)		6.52 (0.95)
	% of control[a]	–		3%		7%		11%

Reference and study design	Results
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Other hormone measurements** Exposure-related changes were not found for progesterone, LH, or estradiol in the F0 and F1 females.
{Maranghi, 2013, 1927558@@author-year} Mice, BALB/c Females only Diet 28-d exposure starting on PND 26	**Doses** (mg/kg-d) **0** / **199** **Testosterone** (ng/mL) **Female** (n = 10) Mean (SD): 0.07 (0.02) / 0.11* (0.07) % of control[a]: – / 57% **Testosterone/estradiol** **Female** (n = 10) Mean (SD): 8.5 (2.1) / 13.3* (6.7) % of control[a]: – / 56% **Other hormone measurements** Exposure-related changes were not found for estradiol.
Reproductive differentiation and development	
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d) **F1 offspring[d]**: **0** / **17** / **168** / **1,570** **F2 offspring[d]**: **0** / **15** / **139** / **1,360** **Time to vaginal opening** (d) **Female F1** (n = 24) Mean (SD): **30.9 (2.0)** / **30.3 (2.6)** / **30.1 (1.8)** / **30.8 (2.2)** % of control[a]: - / **-2%** / **-3%** / **0%** **AGD (mm)** No dose-related changes in the F1 or F2 female pups
{van der Ven, 2009, 589273@@author-year}	**Doses** (mg/kg-d) **0** / **0.1** / **0.3** / **1** / **3** / **10** / **30** / **100** **Time to vaginal opening** (days)

Reference and study design	Results							
Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Female, F1** (n = 4–5)[b] **							
	Mean (SD)	35.4 (2.3)	35.3 (2.2)	36.2 (2.4)	36.8 (4.1)	36.8 (3.3)	35.4 (2.7)	34.8 (1.6)	39.9 (2.6)
	% of control[a]	–	0%	2%	4%	4%	0%	−2%	13%
	AGD (mm)								
	No significant dose-response trend								
{Saegusa, 2009, 787721@@author-year} Crj:CD(SD)IGS, rat Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	**Doses** (mg/kg-d)[c]							
		0		**15**		**146**		**1,505**
	Time to vaginal opening (d)							
	Female F1 (n = 12–14)							
	Mean (SD)	35.4 (1.9)		35.6 (1.8)		34.9 (1.7)		34.4 (2.1)
	% of control[a]	–		1%		−1%		−3%
	AGD (mm)							
	No dose-related change							
Reproductive organ weights								
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d)							
	F1 offspring[d]	**0**		**17**		**168**		**1,570**
	Female F1 adult	**0**		**14**		**138**		**1,363**
	F2 offspring[d]	**0**		**15**		**139**		**1,360**
	Absolute ovary weight (mg)							
	Female, F1, PND 26 (n = 14–23)							
	Mean (SD)	20.8 (3.7)		22.8 (3.6)		21.0 (4.0)		20.9 (3.4)
	% of control[a]	–		10%		1%		0%
	Female, F1, adult (n = 13–22)							
	Mean (SD)	102.4 (12.9)		106.4 (13.2)		108.6 (18.0)		104.9 (16.9)
	% of control[a]	–		4%		6%		2%
	Female, F2, PND 26 (n = 13–21)							
	Mean (SD)	20.0 (3.9)		22.9* (2.6)		20.9 (3.9)		18.2 (4.0)
	% of control[a]	–		14%		4%		−9%
	Relative ovary weight (mg/100 g BW)							
	Female, F1, PND 26 (n = 14–23)							
	Mean (SD)	26.5 (4.5)		27.5 (4.1)		25.0 (3.8)		28.9 (3.7)
	% of control[a]	–		4%		−6%		9%

Reference and study design	Results							
	Female, F1, adult (n = 13–22)							
	Mean (SD)	31.8 (4.2)	32.6 (3.9)	33.1 (5.3)	34.1 (4.2)			
	% of control[a]	–	3%	4%	7%			
	Female, F2, PND 26 (n = 13–21)							
	Mean (SD)	26.9 (5.1)	30.5* (3.9)	28.8 (4.2)	32.1* (7.5)			
	% of control[a]	–	13%	7%	19%			
	Absolute uterus weight (mg)							
	Female, F1, PND 26 (n = 14–23)							
	Mean (SD)	57.0 (10.9)	62.0 (14.1)	64.1 (18.6)	51.9 (12.4)			
	% of control[a]	–	9%	12%	−9%			
	Female, F1, adult (n = 13–22)							
	Mean (SD)	966 (216)	913 (188)	955 (204)	949 (156)			
	% of control[a]	–	−5%	−1%	−2%			
	Female, F2, PND 26 (n = 13–21)							
	Mean (SD)	60.8 (16.1)	63.6 (15.1)	57.0 (15.7)	47.6* (11.4)			
	% of control[a]	–	5%	−6%	−22%			
	Relative uterus weight (mg/100 g BW)							
	Female, F1, PND 26 (n = 14–23)							
	Mean (SD)	73.6 (17.5)	74.9 (17.7)	76.0 (18.4)	71.9 (16.2)			
	% of control[a]	–	2%	3%	−2%			
	Female, F1, adult (n = 13–22)							
	Mean (SD)	299 (64)	282 (65)	291 (64)	313 (69)			
	% of control[a]	–	−6%	−3%	5%			
	Female, F2, PND 26 (n = 13–21)							
	Mean (SD)	80.9 (16.3)	84.4 (21.0)	78.7 (21.7)	83.7 (20.3)			
	% of control[a]	–	4%	−3%	3%			
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Absolute ovary weight (left and right) (g)								
	Female, F1, PNW 11 (n = 4–5)								
	Mean (SD)	0.10 (0.01)	0.13 (0.02)	0.11 (0.02)	0.11 (0.003)	0.13 (0.02)	0.11 (0.02)	0.12 (0.02)	0.11 (0.02)
	% of control[a]	–	21%	11%	9%	24%	8%	17%	7%
	Absolute uterus weight (g)								
	Female, F1, PNW 11 (n = 4–5)								
	Mean (SD)	0.53 (0.11)	0.60 (0.20)	0.50 (0.11)	0.75 (0.38)	0.71 (0.39)	0.94 (0.28)	0.48 (0.10)	0.49 (0.22)
	% of control[a]	–	13%	−6%	42%	34%	77%	−9%	−8%
	Doses (mg/kg-d)								

Reference and study design	Results			
{WIL Research, 2001, 787787@@author-year} Rats, Crl:CD(SD)IGS BR Gavage 90 d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown		**0**	**100**	**300**	**1,000**
	Absolute ovary with oviduct weight (g)				
	Female (n = 10)				
	Mean (SD)	0.14 (0.03)	0.13 (0.03)	0.13 (0.03)	0.15 (0.02)
	% of control[a]	–	−10%	−9%	3%
	Relative ovary with oviduct weight (g/100 g BW)				
	Female (n = 10)				
	Mean (SD)	0.05 (0.01)	0.05 (0.01)	0.05 (0.01)	0.05 (0.01)
	% of control[a]	–	−8%	−12%	2%
	Absolute uterus with cervix weight (g)				
	Female (n = 10)				
	Mean (SD)	0.81 (0.25)	0.64 (0.16)	0.67 (0.14)	0.62 (0.17)
	% of control[a]	–	−21%	−17%	−23%
	Relative uterus with cervix weight (g/100 g BW)				
	Female (n = 10)				
	Mean (SD)	0.29 (0.07)	0.23 (0.05)	0.22 (0.04)	0.22 (0.07)
	% of control[a]	–	−20%	−21%	−23%
{WIL Research, 1997, 787758@@author-year} Rats, Sprague-Dawley Gavage 28-d exposure starting on ~PNW 6 followed by a 14-d recovery period Recovery data not shown	**Doses** (mg/kg-d)	**0**	**125**	**350**	**1,000**
	Relative ovary with oviduct weight (g/100 g BW)				
	Female (n = 6)				
	Mean (SD)	0.06 (0.0003)	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)
	% of control[a]	-	0%	0%	0%
{Saegusa, 2009, 787721@@author-year} Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	**Doses** (mg/kg-d)[d]	**0**	**15**	**146**	**1,505**
	Relative ovary weight (mg/100 g BW)				
	Female, F1, PND 20 (n = 10)				
	Mean (SD)	32.3 (3.9)	30.9 (4.9)	28.1 (6.3)	28.7 (3.4)
	% of control[a]	–	−4%	−13%	−11%
	Female, F1, PNW 11 (n = 10)				
	Mean (SD)	31.8 (6.1)	32.8 (2.6)	32.2 (5.7)	34.0 (4.8)
	% of control[a]	–	3%	1%	7%
	Relative uterus weight (g/100 g BW)				
	Female, F1, PND 20 (n = 10)				
	Mean (SD)	0.08 (0.01)	0.08 (0.01)	0.08 (0.01)	0.07 (0.01)
	% of control[a]	–	0%	−4%	−9%
	Female, F1, PNW 11 (n = 10)				
	Mean (SD)	0.16 (0.04)	0.15 (0.02)	0.16 (0.02)	0.17 (0.03)
	% of control[a]	–	−6%	0%	6%
	Doses (mg/kg-d)				

Reference and study design	Results		
{Maranghi, 2013, 1927558@@author-year} Mice, BALB/c Females only Diet 28-d exposure starting on PND 26		**0**	**199**
	Absolute uterus weight (g)		
	Female (n = 10–15)		
	Mean (SD)	0.140 (0.051)	0.141 (0.041)
	% of control[a]	–	1%
	Relative uterus weight (%)		
	Female (n = 10–15)		
	Mean (SD)	0.66 (0.24)	0.71 (0.21)
	% of control[a]	–	8%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[b]Exact number of animals examined per dose group was unclear in the published paper.
[c]TWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.
[d]F1 and F2 offspring doses presented as maternal F0 and F1 mean gestational and lactational doses, respectively.

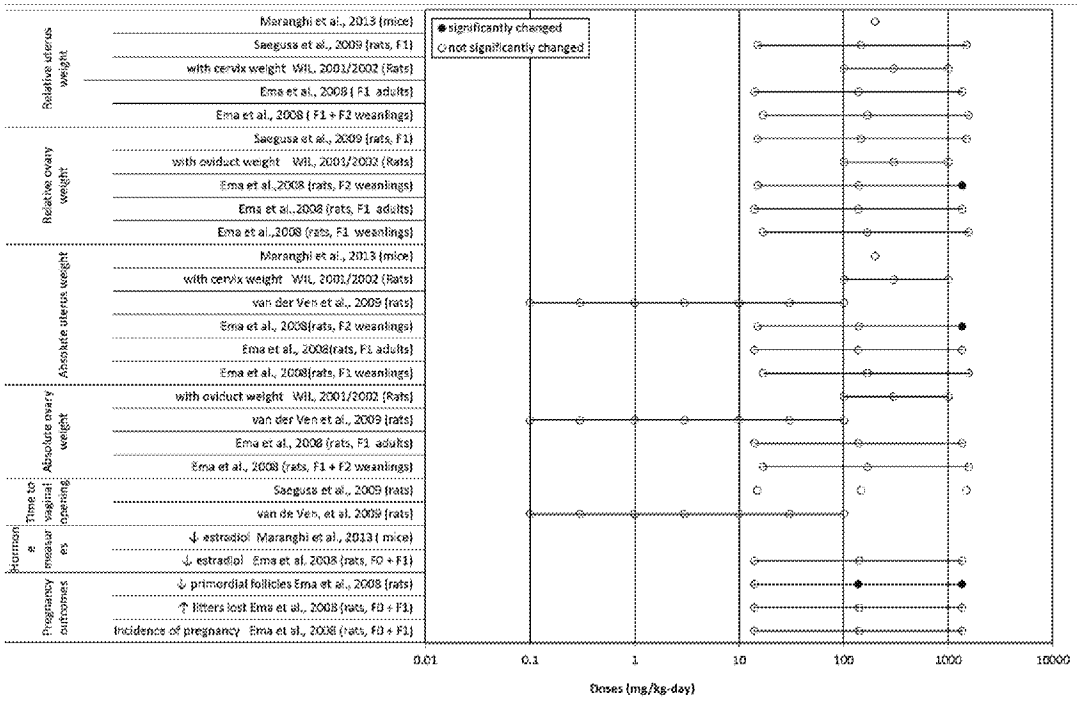


Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Exposure response array of female reproductive system effects following oral exposure.

1.3.1.3 Mechanistic Evidence

The available mechanistic evidence related to HBCD-mediated effects on the reproductive system is focused on dysregulation of reproductive hormone homeostasis.

Human and rodent cell culture models provide some evidence to support the potential for HBCD to alter the function of several reproductive hormones. Human breast cancer cells (MDA-kb2) co-exposed with dihydroxytestosterone, HBCD potentiated expression of androgen-receptor mediated genes, but did not act as a direct AR agonist {Christen, 2010, 697281}. In human prostate cancer cells (LNCaP), however, HBCD treatment elicited a pattern of responses that is characteristic of AR activation (e.g., increased cell migration and viability, and reduction of apoptotic markers), but at a lower potency than the endogenous ligand {Kim, 2016, 3350494}. FSH was also affected in rat granulosa and leydig cells; HBCD altered FSH- and LH-mediated signaling pathways {Fa, 2014, 2343737}{Fa, 2015, 2966753}. Effects on the estrogen receptor are less consistent. Assay findings using human breast cancer cells (T47D and MCF-7) indicated that HBCD may act as an estrogen antagonist {Hamers, 2006, 787675}{Krivoshiev, 2016, 3350477}; however, these findings were not consistent with other studies that

used one of the same breast cancer cell lines (MCF-7) or ovarian cancer cells {Yamada-Okabe, 2005, 787752;Dorosh, 2011, 787655;Kang, 2012, 1401118;Park, 2012, 1249955}.

In addition to hormone receptor level effects, several studies indicate that HBCD may also perturb enzymes involved in the synthesis and metabolism of reproductive hormones. In female rats, HBCD exposure increased mRNA and protein levels as well as activity of the CYP3A family of enzymes {Germer, 2006, 787665;Cantón, 2008, 787647}, which play an important role in the metabolism and excretion of estrogens {Kretschmer, 2005, 1416994}. Studies in rat primary Leydig and human adrenocortical carcinoma cell lines indicate that HBCD exposure may interfere with activity and/or cell signaling pathways of several enzymes involved in steroid synthesis {Scott, 2009, 673313;Cantón, 2006, 1927790}, including CYP17 {Fernandez Canton, 2005, 1717275;Fa, 2013, 1927564} and CYP19A1 {van den Dungen, 2016, 2850361}, CYP11A1, and HSD17β {Fa, 2015, 2966753}.

1.3.2. Male Reproductive Effects

1.3.2

1.3.2.1 Human Evidence

Epidemiological studies evaluating HBCD exposure and reproductive endpoints include a birth cohort {Meijer, 2012, 1401499} and a cross-sectional study of male infertility patients {Johnson, 2013, 1676758} (Table C-1). The birth cohort study in the Netherlands examined maternal serum HBCD levels in relation to male infants' testes volume and penile length at 3 and 18 months (n = 44) as well as steroidal and gonadotropin hormone levels at 3 months (n = 34) {Meijer, 2012, 1401499}. Effect estimates for the association with testes volume or penile length were not provided, but were reported to be not statistically significant. A weak to moderate correlation coefficient ($r = -0.31$; $0.05 < p < 0.10$) was observed between maternal serum HBCD and free testosterone. No other effects on steroidal or gonadotropin hormones were associated with serum HBCD levels (effect estimates not provided). A study examining the relationship between HBCD concentrations in household dust and reproductive hormones in 38 adult men from the United States attending an infertility clinic {Johnson, 2013, 1676758} reported statistically significant correlations for decreased sex hormone binding globulin (SHBG) ($r = -0.35$; $p = 0.03$) and increased free androgen index (testosterone/SHBG) ($r = 0.46$; $p = 0.004$); the effect on the free androgen index was likely due to decreased SHBG levels, as testosterone concentrations did not appear to be related to HBCD exposure. Correlation coefficients for other hormones were not reported, but were described as not statistically significant {Johnson, 2013, 1676758}.

Overall, given the limited evidence for male reproductive effects associated with HBCD exposure and the low confidence in the two studies that evaluated male reproductive outcomes

(see Table C-1), the database was considered inadequate to draw conclusions regarding the relationship between HBCD exposure and male reproductive effects in humans.

1.3.2.2 Animal Evidence

Evidence to inform the potential for HBCD to induce male reproductive effects, including reproductive differentiation and development, spermatogenic measures, and reproductive organ weights, comes from five studies in rats {Ema, 2008, 787657;Saegusa, 2009, 787721;van der Ven, 2009, 589273;WIL Research, 2001, 787787;van der Ven, 2006, 787745} with exposure durations ranging from 28 days to two generations. Evidence pertaining to male reproductive effects in experimental animals following oral exposure to HBCD is summarized in Table C-2 and Figure C-2. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. If not otherwise indicated, endpoint measurements were made in adults.

The available evidence for an association between HBCD exposure and male reproductive effects in experimental animals is inconclusive (Table C-1). One study found a significant dose-related increase in AGD, a measure of reproductive differentiation and development, only on PND 4 {van der Ven, 2009, 589273} and the biological significance of increased AGD is unclear. {van der Ven, 2009, 589273@@author-year} also reported a significant trend with dose for epididymal sperm with separate heads in rats continuously exposed to HBCD from gestation through PNW 11, but not after a 28-day exposure in adults {van der Ven, 2006, 787745}. Statistically significant increases (9–12% relative to control) in relative testis weight were reported for PND 26 F1 rats in all three dose groups (approximately 17−1,500 mg/kg-day) in a two-generation reproductive study {Ema, 2008, 787657}, but not in 15-week F1 males or PND 26 F2 males in the same study. Relative testes weights in HBCD-exposed rats were increased (6–7%) in {WIL Research, 2001, 787787@@author-year} and decreased (4–7%) in {Saegusa, 2009, 787721@@author-year}; in both studies, changes were not statistically significantly different. Two studies reported statistically significant changes in relative prostate weight in high-dose animals; however, the direction of the effect was not consistent across studies, with {Ema, 2008, 787657@@author-year} reporting a decrease and {WIL Research, 2001, 787787@@author-year} reporting an increase. Furthermore, this effect was no longer present following a 4-week recovery period {WIL Research, 2001, 787787}. No other dose-related effects were observed for other measures of male reproductive differentiation and development {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721}, spermatogenic measures {Ema, 2008, 787657;van der Ven, 2006, 787745;van der Ven, 2009, 589273;WIL Research, 2001, 787787}, or male reproductive organ weights {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721;WIL Research, 2001, 787787}.

Table C-1. Evidence pertaining to male reproductive toxicity of HBCD in humans

Reference and study design	Results
{Meijer, 2012, 1401499@@author-year} (the Netherlands, COMPARE cohort, 2001–2002) **Population:** Birth cohort, 90 singleton, term births, 55 healthy boys, assessed at 3 mo (n = 55) and 18 mo (n = 52); 44 with HBCD measures, 45 with hormone measures, 34 with both measures **Exposure measures:** Prenatal exposure, maternal serum at 35th week of pregnancy 1,2,5,6,9,10-HBCD (HBCD) detected in 43 of 44 samples LOD 0.8 pg/g serum; LOQ = 9 pg/g serum Median 0.7 (range: <LOD–7.4) ng/g lipid **Effect measures:** Reproductive hormones (serum, collected at 3 mo) {immunoassay details in \Laven, 2004, 2238548} • testosterone • SHBG • FSH • LH • estradiol • inhibin B Testes volume, measured by ultrasound (ages 3 and 18 mo); penile length (ages 3 and 18 mo) **Analysis:** Spearman correlation **Study evaluation[a]:** [EMBED PBrush] Limited analysis and inadequate reporting of results; small sample size	Spearman correlation between HBCD in maternal serum and free testosterone: r = −0.31 ($0.05 < p$-value < 0.10). Correlations with other hormones noted as not statistically significant, but effect estimates were not reported. No significant correlations between prenatal exposure to HBCD and testes volume or penile length were found (data not shown).
{Johnson, 2013, 1676758@@author-year} (USA, 2002–2003) **Population:** 38 men (18–54 yrs old), from couples seeking infertility treatment; approximately 65% participation into general study; participation rate in the vacuum bag collection phase not reported **Exposure measures:** HBCD exposure from vacuum bag dust; three main stereoisomers of HBCD presented together; HBCD detected in 97% of samples; LOD not reported; median 246 ng/g dust (90th percentile 1,103 ng/g dust) **Effect measures:** Non-fasting blood sample {immunoassay details in \Meeker, 2008, 2238550} testosterone SHBG FSH LH estradiol inhibin B prolactin	Spearman r (p-value) Free androgen index (testosterone/SHBG) 0.46 ($p = 0.004$) SHBG −0.35[a] ($p = 0.03$) Multivariate models adjusted for age and BMI reportedly produced similar results to the bivariate results (data not reported for HBCD). Results for other hormones not shown. Note that HBCD was not strongly correlated with other flame retardants measured (Spearman correlation coefficients ranging from −0.20 to 0.27, all p-values > 0.10)

Reference and study design	Results
Analysis: All variables analyzed as continuous variables; Spearman's correlation between HBCD in house dust and serum hormone levels; multivariable models adjusted for age and BMI, but results for HBCD model results not reported **Study evaluation[a]:** [EMBED PBrush] Limited analysis and inadequate reporting of results; small sample size	

[a]Evaluation of sources of bias or study limitations (see Systematic Review Methods/Epidemiology Studies, and Appendix B, Table B-3): P = population selection; E = exposure misclassification; O = outcome misclassification; C = confounding; A = analysis; Oth = other feature affecting interpretation of results. Extent of column shading reflects degree of limitation.

Table C-2. Evidence pertaining to male reproductive effects in animals following exposure to HBCD

Reference and study design	Results							
Reproductive differentiation and development								
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d)							
	F1 offspring[a]	**0**	**17**	**168**	**1,570**			
	F2 offspring[a]	**0**	**15**	**139**	**1,360**			
	AGD (mm)							
	Male, F1, PND 4 (n = 18–24 litters)							
	Mean (SD)	5.37 (0.41)	5.44 (0.36)	5.38 (0.32)	5.20 (0.51)			
	% change[b]	–	1%	0%	−3%			
	Male, F2, PND 4 (n = 19–22 litters)							
	Mean (SD)	5.12 (0.54)	5.12 (0.41)	5.04 (0.42)	4.84 (0.39)			
	% change[b]	–	0%	−2%	−5%			
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating	**Doses** (mg/kg-d)							
		0	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	AGD (mm)								
	Male, F1, PND 4 (n ≥ 14)[c] **								
	Mean (SD)	4.6 (0.8)	5.1 (1.1)	4.7 (0.8)	4.8 (1.0)	5.0 (0.8)	5.0 (0.9)	4.5 (0.8)	5.4 (1.0)
	% change[b]	–	11%	2%	4%	9%	9%	−2%	17%
	Male, F1, PND 7 (n ≥ 14)[c]								
	Mean (SD)	6.2 (1.2)	6.7 (1.2)	5.5 (1.1)	6.4 (1.4)	6.1 (1.3)	6.0 (1.3)	6.6 (1.0)	6.3 (1.2)
	% change[b]	–	8%	−11%	3%	−2%	−3%	6%	2%
	Male, F1, PND 21 (n ≥ 14)[c]								

Reference and study design	Results							
F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	Mean (SD)	19.0 (6.0)	19.1 (4.1)	14.8 (2.6)		18.7 (2.9)	18.3 (5.5)	18.9 (6.1)	16.0 (2.2)
	% change[b]	–	1%	−22%	n/a	−2%	−4%	−1%	−16%
	Value for male F1 PND 21 rats at 1 mg/kg-d was "n/a" in study report.								
{Saegusa, 2009, 787721@@author-year} Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	**Doses** (mg/kg-d)[d]								
	0		**15**		**146**		**1,505**		
	AGD (mm)								
	Male, F1, PND 1 (n = 10 litters)								
	Mean (SD)	3.88 (0.23)		3.96 (0.20)		4.08 (0.30)		4.01 (0.23)	
	% change[b]	–		2%		5%		3%	
Spermatogenic measures									
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)								
	0	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**	
	Epididymal sperm with separate heads (% of total)								
	Male, F1, PNW 11 (n = 4–5)**								
	Mean (SD)	4.2 (1.7)	3.8 (2.9)	7.5 (8.1)	2.2 (1.9)	4.4 (1.9)	4.1 (2.1)	5.0 (1.8)	0.8 (0.8)
	% change[b]	–	−10%	79%	−48%	5%	−2%	19%	−81%
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure starting on PNW 11	**Doses** (mg/kg-d)								
	0	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**	
	Epididymal sperm with separate heads (% of total)								
	Male (n = 4–5)								
	Mean (SD)	5.3 (2.9)	3.8 (2.2)	7.4 (3.2)	4.7 (3.4)	5.1 (4.0)	6.8 (4.1)	3.5 (2.7)	5.1 (3.6)
	% change[b]	–	−28%	40%	−11%	−4%	28%	−34%	−4%
Reproductive organ weights									
{Ema, 2008, 787657@@author-year}	**Doses** (mg/kg-d)								
	F1, offspring[a]	**0**		**17**		**168**		**1,570**	
	Male, F1, adult	**0**		**11**		**115**		**1,142**	

Reference and study design	Results							
Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**F2, offspring**[a]	**0**	**15**	**139**	**1,360**			
	Relative epididymis weight (left and right) (mg/100 g BW)							
	Male, F1, PND 26 (n = 17–23)							
	Mean (SD)	85.9 (9.8)	86.7 (10.3)	89.3 (7.5)	89.9 (15.3)			
	% change[b]	–	1%	4%	5%			
	Male, F1 adult (n = 22–24)							
	Mean (SD)	223 (24)	232 (24)	210 (19)	234 (23)			
	% change[b]	–	4%	−6%	5%			
	Male, F2, PND 26 (n = 13–22)							
	Mean (SD)	90.7 (14.1)	87.2 (10.6)	87.3 (9.6)	96.2 (10.5)			
	% change[b]	–	−4%	−4%	6%			
	Relative testis weight (left and right) (mg/100 g BW)							
	Male, F1, PND 26 (n = 17–23)							
	Mean (SD)	0.57 (0.07)	0.61* (0.06)	0.62* (0.06)	0.63* (0.07)			
	% change[b]	–	9%	9%	12%			
	Male, F1 adult (n = 22–24)							
	Mean (SD)	0.60 (0.07)	0.61 (0.05)	0.58 (0.06)	0.59 (0.07)			
	% change[b]	–	2%	−4%	−1%			
	Male, F2, PND 26 (n = 13–22)							
	Mean (SD)	0.57 (0.01)	0.60 (0.06)	0.57 (0.09)	0.59 (0.05)			
	% change[b]	–	5%	0%	3%			
	Relative ventral prostate weight (mg/100 g BW)							
	Male, F1, PND 26 (n = 17–23)							
	Mean (SD)	46.4 (10.3)	47.1 (8.8)	48.2 (7.3)	44.5 (11.1)			
	% change[b]	–	2%	4%	−4%			
	Male, F1 adult (n = 22–24)							
	Mean (SD)	137 (28)	135 (34)	131 (30)	135 (22)			
	% change[b]	–	−1%	−4%	−1%			
	Male, F2, PND 26 (n = 13–22)							
	Mean (SD)	50.2 (9.3)	50.2 (10.7)	50.8 (9.6)	47.3 (15.8)			
	% change[b]	–	0%	1%	−6%			
{van der Ven, 2009, 589273@@author-year} Rats, Wistar	**Doses** (mg/kg-d)							
	Male, F1	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Absolute epididymis weight (left and right) (g)								
	Male, F1, PNW 11 (n = 4–5)								

Reference and study design	Results								
Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	Mean (SD)	0.95 (0.13)	0.88 (0.13)	0.95 (0.12)	1.00 (0.06)	0.90 (0.09)	0.85 (0.13)	0.98 (0.14)	0.82 (0.06)
	% change[b]	–	−7%	0%	5%	−5%	−11%	3%	−14%
	Absolute testis weight (left and right) (g)								
	Male, F1, PNW 11 (n = 4–5)**								
	Mean (SD)	3.01 (0.17)	2.91 (0.08)	3.07 (0.42)	3.18 (0.20)	2.88 (0.28)	2.82 (0.07)	2.97 (0.25)	2.60 (0.06)
	% change[b]	–	−3%	2%	6%	−4%	−6%	−1%	−14%
	Absolute prostate weight (g)								
	Male, F1, PNW 11 (n = 4–5)**								
	Mean (SD)	0.66 (0.18)	0.73 (0.21)	0.57 (0.15)	0.73 (0.21)	0.57 (0.12)	0.58 (0.07)	0.67 (0.09)	0.42 (0.13)
	% change[b]	–	11%	−14%	11%	−14%	−12%	2%	−36%
	Absolute seminiferous vesicle weight (g)								
	Male, F1, PNW 11 (n = 4–5)								
	Mean (SD)	1.00 (0.40)	1.07 (0.22)	1.32 (0.23)	1.14 (0.29)	1.21 (0.09)	1.07 (0.29)	1.21 (0.25)	1.09 (0.27)
	% change[b]	–	7%	32%	14%	21%	7%	21%	9%
{WIL Research, 2001, 787787@@author-year} Rats, Crl:CD(SD)IGS BR Gavage 90 d exposure starting on ~PNW 7 followed by a 28-d recovery period Recovery data not shown	**Doses** (mg/kg-d)								
	Male	**0**		**100**		**300**		**1,000**	
	Relative prostate weight (g/100 g BW)								
	Male (n = 9–10)								
	Mean (SD)	0.18 (0.03)		0.19 (0.03)		0.21 (0.04)		0.26 (0.05)	
	% change[b]	–		3%		17%		42%	
	Relative testis weight (left) (g/100 g BW)								
	Male (n = 9–10)								
	Mean (SD)	0.30 (0.08)		0.31 (0.04)		0.31 (0.04)		0.32 (0.04)	
	% change[b]	–		4%		2%		7%	
	Relative testis weight (right) (g/100 g BW)								
	Male (n = 9–10)								
	Mean (SD)	0.31 (0.07)		0.31 (0.04)		0.31 (0.04)		0.32 (0.05)	
	% change[b]	–		0%		1%		6%	
	Relative cauda epididymis weight (left) (g/100 g BW)								
	Male (n = 9–10)								
	Mean (SD)	0.05 (0.01)		0.06 (0.01)		0.06 (0.01)		0.06 (0.01)	
	% change[b]	–		9%		6%		15%	

Reference and study design	Results				
	Relative cauda epididymis weight (right) (g/100 g BW)				
	Male (n = 9–10)				
	Mean (SD)	0.05 (0.01)	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)
	% change[b]	–	6%	4%	17%
	Relative epididymis weight (left) (g/100 g BW)				
	Male (n = 9–10)				
	Mean (SD)	0.12 (0.02)	0.13 (0.01)	0.12 (0.02)	0.14 (0.01)
	% change[b]	–	8%	3%	13%
	Relative epididymis weight (right) (g/100 g BW)				
	Male (n = 9–10)				
	Mean (SD)	0.12 (0.04)	0.13 (0.01)	0.13 (0.01)	0.14 (0.02)
	% change[b]	–	8%	3%	16%
{Saegusa, 2009, 787721@@author-year} Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	**Doses** (mg/kg-d)[d]				
	Male, F1	**0**	**14.8**	**146.3**	**1,505**
	Relative epididymis weight (left and right) (g/100 g BW)				
	Male, F1, PND 20 (n = 10)				
	Mean (SD)	0.06 (0.02)	0.07 (0.01)	0.07 (0.01)	0.07 (0.01)
	% change[b]	–	8%	13%	8%
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.23 (0.02)	0.21* (0.01)	0.22 (0.02)	0.21 (0.01)
	% change[b]	–	−9%	−4%	−9%
	Relative testis weight (left and right) (g/100 g BW)				
	Male, F1, PND 20 (n = 10)				
	Mean (SD)	0.43 (0.04)	0.43 (0.03)	0.43 (0.05)	0.40 (0.03)
	% change[b]	–	0%	0%	−7%
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.77 (0.07)	0.73 (0.04)	0.78 (0.09)	0.74 (0.05)
	% change[b]	–	−5%	1%	−4%
	Relative dorsolateral prostate weight (mg/100 g BW)				
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.13 (0.03)	0.13 (0.01)	0.14 (0.03)	0.13 (0.02)
	% change[b]	–	0%	8%	0%
	Relative ventral prostate weight (mg/100 g BW)				
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.13 (0.02)	0.13 (0.04)	0.12 (0.03)	0.12 (0.01)

Reference and study design	Results				
	% change[b]	–	0%	−8%	−8%
	Relative seminal vesicle weight (mg/100 g BW)				
	Male, F1 adult, PNW 11 (n = 10)				
	Mean (SD)	0.27 (0.05)	0.26 (0.03)	0.26 (0.05)	0.26 (0.05)
	% change[b]	–	−4%	−4%	−4%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]F1 and F2 offspring doses presented as mean maternal gestational and lactational F0 and F1 doses, respectively.
[b]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[c]Exact number of animals examined per dose group was unclear in the published paper.
[d]TWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PND 1–9, and PND 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.

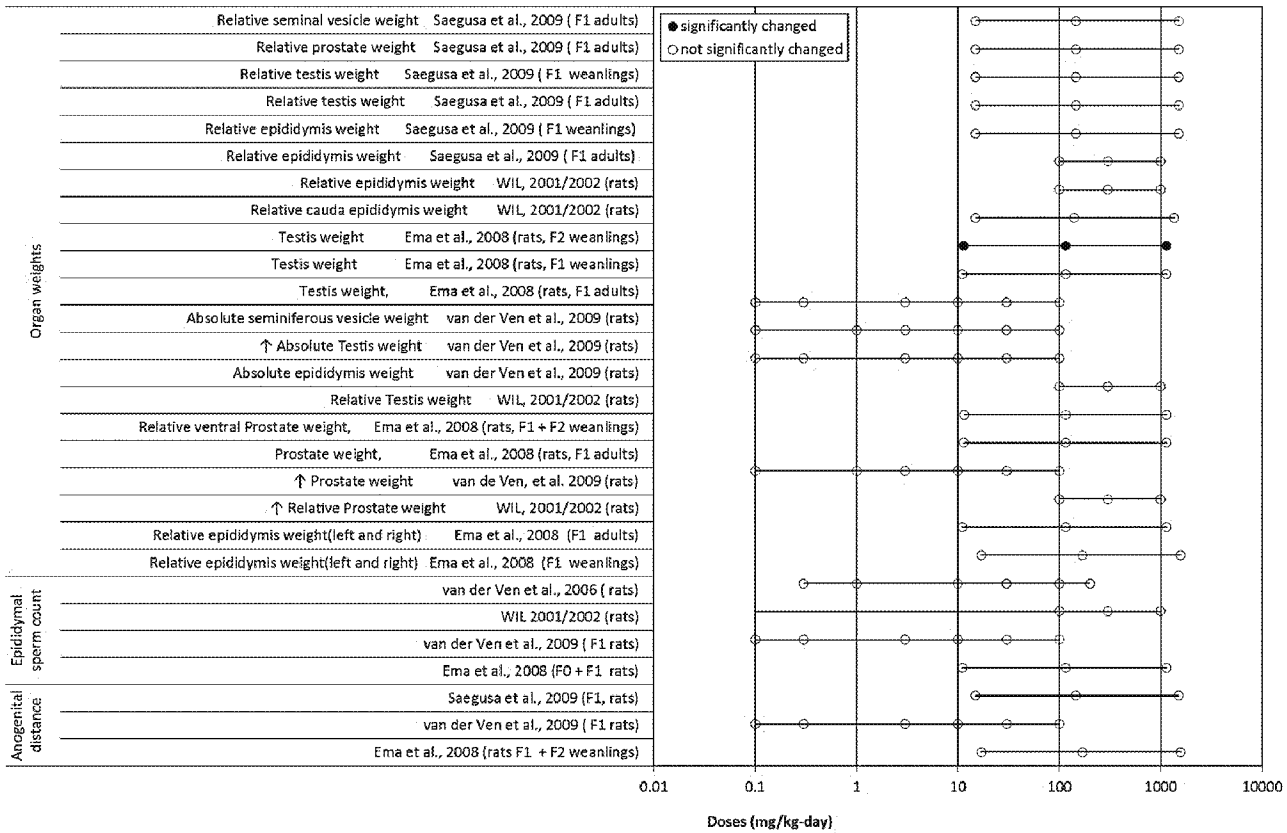


Figure C-2. Exposure response array of male reproductive system effects following oral exposure.

Commented [A1]: New ER arrays are housed in HAWC.

1.3.2.3 Mechanistic Evidence

See Section [REF _Ref532817803 \n \h * MERGEFORMAT] in the Female Reproductive Effects section above (Mechanistic Evidence).

1.4. Developmental Effects

1.3.3

1.4.1. Human Evidence

Epidemiology studies investigating potential thyroid, male reproductive, and nervous system effects of HBCD following developmental exposure were identified and are discussed in their respective organ/system-specific hazard sections (Sections [REF _Ref532817872 \n \h * MERGEFORMAT], [REF _Ref532817890 \n \h * MERGEFORMAT], and [REF _Ref532817905 \n \h * MERGEFORMAT], respectively).

1.4.2. Animal Evidence

Evidence to inform organ-system specific effects of HBCD in animals following developmental exposure are discussed in the individual hazard sections. The current section is limited to discussion of developmental specific effects, including offspring survival, pup body weight, developmental markers, and bone measures.

HBCD-induced developmental effects, including offspring survival, body weight, and developmental markers, were evaluated in five studies in rats {Ema, 2008, 787657;Saegusa, 2009, 787721;van der Ven, 2009, 589273;Hachisuka, 2010, 2919532} and mice {Maranghi, 2013, 1927558}, with exposure durations ranging from 28 days in juvenile mice to continuous exposure of rats over two generations. A summary of developmental effects associated with HBCD exposure is presented in [REF _Ref532804158 \h * MERGEFORMAT] and [REF _Ref532817986 \h * MERGEFORMAT]. Effect categories with stronger evidence are presented first, with individual studies ordered by study duration and then species. For each endpoint, age at outcome measurement is indicated.

Effects on offspring survival and pup body weight were evaluated in three rat studies {Ema, 2008, 787657;Saegusa, 2009, 787721;van der Ven, 2009, 589273} and juvenile body weight was reported in a single mouse study {Maranghi, 2013, 1927558}. Two rat studies that utilized similar dose ranges (approximately 10–1,500 mg/kg-day) reported statistically significant effects in the high-dose group {Ema, 2008, 787657;Saegusa, 2009, 787721}. {Ema, 2008, 787657@@author-year} reported decreases in pup body weight ranging from 20 to 25% for male and female F2 rat pups on PNDs 7, 14, and 21. Offspring survival on PNDs 4 and 21 (21 and 42%, respectively) in this dose group was also decreased {Ema, 2008, 787657}. Decreases in pup weight in F1 animals were smaller (<10%), did not show a consistent pattern of effect, and were not associated with decreased viability {Ema, 2008, 787657;Saegusa, 2009, 787721}. The remaining studies indicate a potential for HBCD to decrease body weight {van der Ven, 2009, 589273;Maranghi, 2013, 1927558} but not viability {van der Ven, 2009, 589273} at lower doses (up to 199 mg/kg-day). {van der Ven, 2009, 589273@@author-year} reported

significant dose-dependent trends in decreased body weight in male and female rat pups. Similarly, {Maranghi, 2013, 1927558@@author-year} reported a 14% body weight decrease in juvenile female mice exposed for 28 days, although this effect was not statistically significant. Use of a single-dose study design did not allow for evaluation of dose-response in this study.

Treatment-related effects on several developmental landmarks were evaluated in F1 and F2 offspring in the two-generation reproductive toxicity study {Ema, 2008, 787657}. In F1 pups, eye opening on PND 14 was significantly increased in both sexes in the mid-dose group, but not the high-dose group (approximately 170 and 1,500 mg/kg-day, respectively). In contrast, F2 offspring exhibited statistically significant dose-related decreases in eye opening on PND 14 in both the mid- (females only) and high-dose groups (males and females). Other developmental landmarks (i.e., pinna unfolding, and incisor eruption) were not affected {Ema, 2008, 787657}.

Measures of bone development were also evaluated in rats treated continuously from gestation through adulthood at doses up to 100 mg/kg-day {van der Ven, 2009, 589273}. Trabecular bone mineral density in females was decreased by 20%. The study authors reported dose-related decreases in several other tibia related endpoints; however, the magnitude of these effects was small and inconsistent across dose group and sex, making it difficult to interpret the biological significance of these findings.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Evidence pertaining to developmental effects in animals following exposure to HBCD

Reference and study design	Results				
Fetal and early postnatal survival					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d)				
	F1 offspring[a]	**0**	**17**	**168**	**1,570**
	F2 offspring[a]	**0**	**15**	**139**	**1,360**
	Viability index (%)				
	F1, PND 0 (n = 18–24 litters)				
	Mean (SD)	99.6 (1.9)	97.5 (8.5)	98.8 (2.8)	99.2 (2.5)
	% of control[b]	–	−2%	−1%	0%
	F1, PND 4 (n = 18–24 litters)				
	Mean (SD)	95.6 (8.6)	98.7 (2.8)	98.7 (4.4)	95.8 (10.3)
	% of control[b]	–	3%	3%	0%
	F1, PND 21 (n = 18–24 litters)				
	Mean (SD)	93.2 (17.3)	99.4 (2.7)	98.1 (4.6)	93.8 (23.6)
	% of control[b]	–	7%	5%	1%
	F2, PND 0 (n = 20–23 litters)				
	Mean (SD)	98.6 (5.3)	97.7 (4.9)	96.0 (9.5)	97.8 (5.1)
	% of control[b]	–	−1%	−3%	−1%
	F2, PND 4 (pre-culling) (n = 20–23 litters)				
	Mean (SD)	86.9 (24.8)	87.3 (21.1)	92.1 (12.8)	68.4* (33.5)

Reference and study design	Results				
	% of control[b]	–	0%	6%	−21%
	F2, PND 21 (n = 20–22 litters)				
	Mean (SD)	85.0 (22.0)	89.6 (13.9)	71.3 (26.9)	49.7* (41.1)
	% of control[b]	–	5%	−16%	−42%
{Saegusa, 2009, 787721@@author-year} Rats, Crj:CD(SD)IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11	**Doses** (mg/kg-d)[c]				
		0	**15**	**146**	**1,505**
	Number of live pups				
	Female, F0 (n = 10 litters)				
	Mean (SD)	13.0 (1.8)	13.0 (1.6)	11.6 (1.6)	12.9 (1.4)
	% of control[b]	–	0%	−11%	−1%
Body weight					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d)				
	F1 offspring[a]	**0**	**17**	**168**	**1,570**
	F2 offspring[a]	**0**	**15**	**139**	**1,360**
	Pup weight (g)				
	Male, F1, PND 0 (n = 18–24 litters)				
	Mean (SD)	6.8 (0.5)	6.9 (0.6)	7.2 (0.7)	6.8 (0.6)
	% of control[b]	–	1%	6%	0%
	Male, F1, PND 4 (n = 18–24 litters)				
	Mean (SD)	10.2 (1.7)	10.7 (1.8)	10.8 (1.6)	9.5 (1.8)
	% of control[b]	–	5%	6%	−7%
	Male, F1, PND 7 (n = 17–24 litters)				
	Mean (SD)	16.4 (3.1)	17.5 (2.4)	16.9 (2.2)	15.6 (2.0)
	% of control[b]	–	7%	3%	−5%
	Male, F1, PND 14 (n = 17–23 litters)				
	Mean (SD)	36.1 (4.8)	36.3 (3.6)	36.1 (3.9)	33.5 (2.6)
	% of control[b]	–	1%	0%	−7%
	Male, F1, PND 21 (n = 17–23 litters)				
	Mean (SD)	61.1 (7.1)	62.3 (6.5)	61.9 (6.5)	55.4* (4.0)
	% of control[b]	–	2%	1%	−9%
	Female, F1, PND 0 (n = 18–23 litters)				
	Mean (SD)	6.3 (0.5)	6.6 (0.7)	6.8* (0.6)	6.5 (0.7)
	% of control[b]	–	5%	8%	3%
	Female, F1, PND 4 (n = 18–23 litters)				
	Mean (SD)	9.6 (1.4)	10.3 (1.8)	10.4 (1.5)	9.2 (1.6)
	% of control[b]	–	7%	8%	−4%
	Female, F1, PND 7 (n = 17–23 litters)				
	Mean (SD)	15.4 (2.8)	17.0 (2.5)	16.9 (2.3)	15.1 (1.6)

Reference and study design	Results							
	% of control[b]	–	10%	10%	−2%			
	Female, F1, PND 14 (n = 17–23 litters)							
	Mean (SD)	33.5 (5.3)	35.5 (3.6)	35.7 (3.6)	32.6 (3.0)			
	% of control[b]	–	6%	7%	−3%			
	Female, F1, PND 21 (n = 17–23 litters)							
	Mean (SD)	56.5 (8.0)	59.9 (6.4)	60.5 (5.9)	53.2 (4.7)			
	% of control[b]	–	6%	7%	−6%			
	Male, F2, PND 0 (n = 20–23 litters)							
	Mean (SD)	6.8 (0.8)	6.7 (0.7)	7.1 (0.6)	6.6 (0.6)			
	% of control[b]	–	−1%	4%	−3%			
	Male, F2, PND 4 (n = 19–22 litters)							
	Mean (SD)	9.1 (2.3)	9.3 (1.3)	9.0 (1.8)	8.0 (1.3)			
	% of control[b]	–	2%	−1%	−12%			
	Male, F2, PND 7 (n = 17–22 litters)							
	Mean (SD)	14.7 (3.9)	15.4 (2.8)	14.3 (3.6)	11.5* (2.9)			
	% of control[b]	–	5%	−3%	−22%			
	Male, F2, PND 14 (n = 14–22 litters)							
	Mean (SD)	31.4 (8.0)	33.8 (5.0)	31.0 (7.2)	24.2* (6.6)			
	% of control[b]	–	8%	−1%	−23%			
	Male, F2, PND 21 (n = 13–22 litters)							
	Mean (SD)	53.0 (12.6)	56.2 (6.7)	54.1 (10.1)	42.6* (8.3)			
	% of control[b]	–	6%	2%	−20%			
	Female, F2, PND 0 (n = 20–23 litters)							
	Mean (SD)	6.5 (0.8)	6.3 (0.6)	6.7 (0.6)	6.2 (0.6)			
	% of control[b]	–	−3%	3%	−5%			
	Female, F2, PND 4 (n = 20–22 litters)							
	Mean (SD)	8.9 (2.3)	8.5 (1.3)	8.8 (1.8)	7.3* (1.3)			
	% of control[b]	–	−5%	−1%	−22%			
	Female, F2, PND 7 (n = 17–22 litters)							
	Mean (SD)	14.3 (3.5)	14.2 (2.8)	13.5 (3.9)	10.7* (2.6)			
	% of control[b]	–	−1%	−6%	−25%			
	Female, F2, PND 14 (n = 13–22 litters)							
	Mean (SD)	31.2 (6.5)	31.3 (5.1)	29.3 (7.3)	23.9* (5.9)			
	% of control[b]	–	0%	−6%	−23%			
	Female, F2, PND 21 (n = 13–22 litters)							
	Mean (SD)	52.0 (10.0)	52.8 (6.6)	51.2 (10.8)	41.6* (8.4)			
	% of control[b]	–	2%	−2%	−20%			
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet	**Doses** (mg/kg-d)							
	0	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Pup weight (g)							
	Male, F1, PND 4 (n ≥ 14)[d] **							

Reference and study design	Results								
One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	Mean (SD)	10.0 (1.3)	10.2 (0.7)	9.8 (1.2)	10.8 (1.9)	10.2 (1.7)	10.8 (1.4)	11.0 (1.3)	9.5 (0.9)
	% of control[b]	–	2%	−2%	8%	2%	8%	10%	−5%
	Male, F1, PND 7 (n ≥ 14)[d]								
	Mean (SD)	13.4 (2.2)	13.6 (1.6)	12.7 (2.0)	14.7 (4.1)	13.1 (3.0)	13.9 (2.7)	14.6 (1.7)	12.6 (1.0)
	% of control[b]	–	1%	−5%	10%	−2%	4%	9%	−6%
	Male, F1, PND 14 (n ≥ 14)[d] **								
	Mean (SD)	22.3 (6.4)	24.2 (5.0)	22.0 (4.0)	33.3 (8.6)	24.1 (7.7)	24.6 (6.5)	22.5 (3.2)	20.5 (2.2)
	% of control[b]	–	9%	−1%	49%	8%	10%	1%	−8%
	Male, F1, PND 21 (n ≥ 14)[d] **								
	Mean (SD)	39.3 (7.5)	41.8 (8.9)	35.1 (5.2)	55.7 (14.4)	39.1 (12.0)	39.5 (10.0)	35.6 (6.2)	32.2 (3.0)
	% of control[b]	–	6%	−11%	42%	−1%	1%	−9%	−8%
	Female, F1, PND 4 (n ≥ 14)[d] **								
	Mean (SD)	9.5 (1.5)	9.7 (0.8)	9.4 (1.1)	10.6 (2.7)	9.4 (1.5)	10.8 (1.1)	10.7 (1.2)	8.9 (0.9)
	% of control[b]	–	2%	−1%	12%	−1%	14%	13%	−6%
	Female, F1, PND 7 (n ≥ 14)[d] **								
	Mean (SD)	12.9 (2.6)	12.8 (1.4)	12.4 (2.1)	14.2 (5.1)	12.5 (2.7)	14.4 (2.2)	14.1 (1.7)	11.9 (1.3)
	% of control[b]	–	−1%	−4%	10%	−3%	12%	9%	−8%
	Female, F1, PND 14 (n ≥ 14)[d] **								
	Mean (SD)	23.6 (5.3)	23.1 (2.7)	21.0 (3.8)	31.1 (7.9)	22.4 (6.0)	24.7 (5.8)	22.5 (4.4)	20.0 (2.9)
	% of control[b]	–	−2%	−11%	32%	−5%	5%	−5%	−15%
	Female, F1, PND 21 (n ≥ 14)[d] **								
	Mean (SD)	40.3 (8.6)	40.1 (5.9)	34.1 (5.4)	50.4 (11.9)	37.0 (10.3)	40.0 (9.5)	37.5 (5.9)	32.3 (3.9)
	% of control[b]	–	0%	−15%	25%	−8%	−1%	−7%	−20%
{Hachisuka, 2010, 2919532@@author-year} Rats, Sprague-Dawley Diet F1: maternal exposure from GD 10 to PNW 3 followed by an 8-wk non-exposure period through PNW 11[e]	**Doses** (mg/kg-d)[c]	**0**		**15**		**146**		**1,505**	
	Pup weight (g)								
	Male, F1, PNW 3 (n = 10)								
	Mean (SD)	51.18 (5.95)		56.10 (3.20)		51.87 (5.95)		53.58 (3.20)	
	% of control[b]	–		10%		1%		5%	
	Male, F1, PNW 11 (n = 10)								
	Mean (SD)	418.94 (15.79)		447.55 (27.63)		456.47 (23.68)		429.82 (35.53)	
	% of control[b]	–		7%		9%		3%	
	Data digitized from figure.								
{Saegusa, 2009, 787721@@author-year} Rats, Crj:CD(SD)IGS	**Doses** (mg/kg-d)[c]	**0**		**15**		**146**		**1,505**	
	Pup weight (g)								

Reference and study design	Results				
Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk non-exposure period through PNW 11 [e]	**Male, F1, PND 1** (n = 10 litters)				
	Mean (SD)	7.11 (0.66)	7.22 (0.56)	7.65 (0.95)	7.15 (0.80)
	% of control[b]	–	2%	8%	1%
	Male, F1, PND 20 (n = 10)				
	Mean (SD)	54.3 (3.5)	51.2 (7.3)	56.7 (4.1)	54.0 (3.3)
	% of control[b]	–	−6%	4%	−1%
	Male, F1, at puberty onset ~PND 40 (n = 12−14)				
	Mean (SD)	204.3 (15.7)	198.3 (20.4)	203.2 (15)	195.8 (10.1)
	% of control[b]	–	−3%	−1%	−4%
	Male, F1, PNW 11 (n = 10)				
	Mean (SD)	454.3 (25.4)	456.9 (24.8)	450.8 (33.4)	435.1 (24.6)
	% of control[b]	–	1%	−1%	−4%
	Female, F1, PND 1 (n = 10 litters)[c]				
	Mean (SD)	6.53 (0.59)	6.84 (0.50)	7.28 (0.75)	6.84 (0.81)
	% of control[b]	–	5%	11%	5%
	Female, F1, PND 20 (n = 10)				
	Mean (SD)	50.3 (3.4)	50.0 (6.0)	53.7 (5.5)	51.3 (2.9)
	% of control[b]	–	−1%	7%	2%
	Female, F1, at puberty onset ~PND 35 (n = 12−14)				
	Mean (SD)	130.8 (11.7)	133.8 (10.8)	129.2 (13.5)	118.6* (11.7)
	% of control[b]	–	2%	−1%	−9%
	Female, F1, PNW 11 (n = 10)				
	Mean (SD)	286.2 (25.2)	293.4 (21.5)	289.2 (24.4)	270.7 (19.6)
	% of control[b]	–	3%	1%	−5%
{Maranghi, 2013, 1927558@@author-year} Mice, BALB/c Females only Diet 28-d exposure starting on PND 26	**Doses** (mg/kg-d)		**0**		**199**
	Body weight gain (g)				
	Female, PND 54 (n = 10−15)				
	Mean (SD)		5.80 (0.74)		5.00 (1.16)
	% of control[b]		--		−14%
Developmental markers					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal	**Doses** (mg/kg-d)				
	F1 offspring[a]	**0**	**17**	**168**	**1,570**
	F2 offspring[a]	**0**	**15**	**139**	**1,360**
	Eye opening (%)				
	Male, F1, PND 14 (n = 17−23 litters)				
	Mean (SD)	48.2 (41.5)	56.7 (37.9)	77.1* (36.3)	45.8 (34.6)
	% of control[b]	–	18%	60%	−5%
	Female, F1, PND 14 (n =17−23 litters)				
	Mean (SD)	49.3 (37.8)	66.7 (41.3)	82.9* (33.5)	54.9 (41.4)

Reference and study design	Results							
exposure throughout gestation/lactation	% of control[b]	–	35%		68%		11%	
	Male, F2, PND 14 (n = 14–22 litters)							
	Mean (SD)	72.7 (40.0)	62.5 (40.6)		47.2 (44.8)		33.9* (34.7)	
	% of control[b]	–	−14%		−35%		−53%	
	Female, F2, PND 14 (n = 13–21 litters)							
	Mean (SD)	82.9 (26.8)	72.7 (37.7)		53.8* (40.3)		48.1* (42.0)	
	% of control[b]	–	−12%		−35%		−42%	
	No exposure-related changes were found in incisor eruption (PND 11) or pinna unfolding (PND 3).							
Bone measures								
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Trabecular bone mineral density, tibia (mg/cm^3)								
	Male, F1, PNW 11 (n = 4–5)								
	Mean (SD)	145 (25)	143 (20)	154 (23)	167 (16)	134 (36)	146 (25)	156 (20)	167 (11)
	% of control[b]	–	−1%	6%	15%	−8%	1%	8%	15%
	Female, F1, PNW 11 (n = 5)**								
	Mean (SD)	294 (19)	268 (27)	253 (30)	231 (35)	245 (31)	227 (28)	200 (31)	234 (29)
	% of control[b]	–	−9%	−14%	−21%	−17%	−23%	−32%	−20%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]F1 and F2 offspring doses presented as mean maternal gestational and lactational F0 and F1 doses, respectively.
[b]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[c]TWA doses for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.
[d]Exact number of animals examined per dose group was unclear based on the published paper.
[e]{Saegusa, 2009, 787721@@author-year} and {Hachisuka, 2010, 2919532@@author-year} appear to be two publications of the same animal cohort; the TWA doses calculated for {Saegusa, 2009, 787721@@author-year} were applied to {Hachisuka, 2010, 2919532@@author-year}.

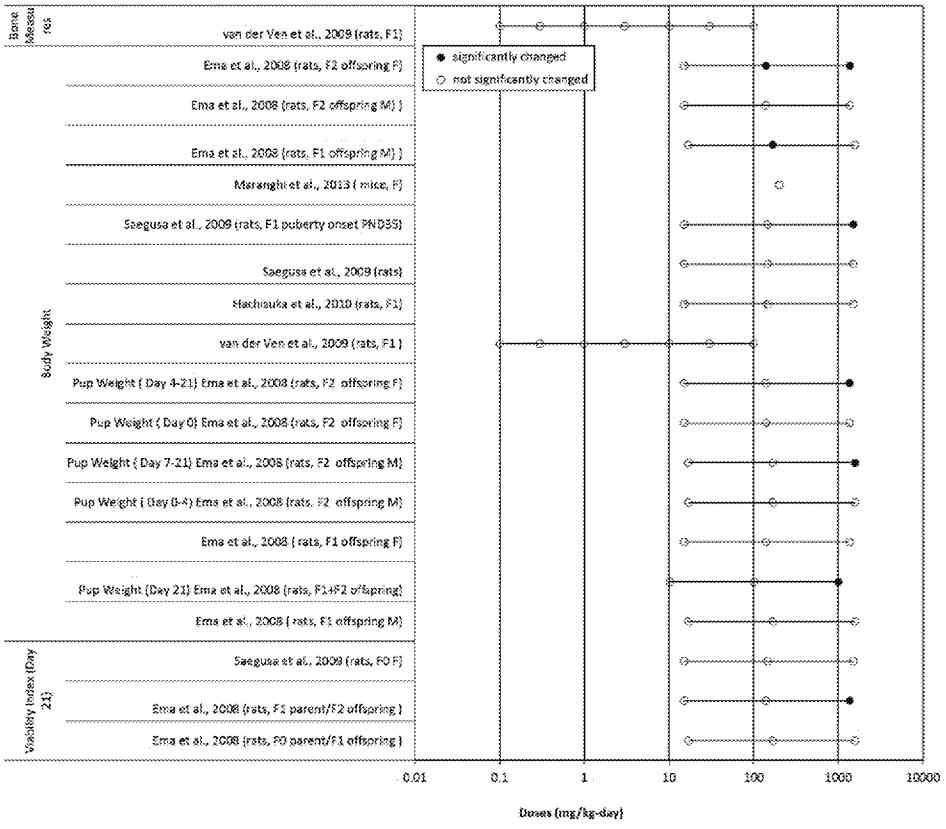


Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Exposure response array of developmental effects following oral exposure.

1.4.3. Mechanistic Evidence

Studies directly investigating mechanistic evidence to inform potential developmental effects of HBCD are limited to a few studies in zebrafish {Wu, 2013, 1927533;Du, 2012, 1927610;Deng, 2009, 1927716;Hu, 2009, 1927732}, which focus on identifying molecular targets that drive HBCD-mediated perturbation of normal embryonic development. In general, HBCD exposure was associated with increased ROS generation and induction of apoptotic cell pathways resulting in malformations and reduced viability in zebrafish {Du, 2012, 1927610;Deng, 2009, 1927716;Hu, 2009, 1927732}. In the absence of overt teratogenic effects, HBCD exposure was found to affect cardiac function and development, resulting in increased heart rate, arrhythmia, cardiac hypertrophy, and increased collagen deposition; these effects were associated with changes in expression of genes associated with calcium transport and cardiomyocyte conduction {Wu, 2013, 1927533}{Wu, 2016, 3350515}. In rat

cardiomyocytes (H9C2), HBCD treatment altered Ca2+ signaling through changes in expression of several genes (Ryr2, Serca2a, and Ncx1) involved in Ca2+ regulation {Wu, 2016, 3350515}.

Although no studies were identified that directly investigated the potential for HBCD-driven thyroid hormone imbalances to induce developmental effects, in vivo studies provide evidence of an association between HBCD exposure and disrupted homeostasis of thyroid hormones (see Section 1.2.1), which are critical regulators of growth and development. In humans, umbilical T4 concentrations are positively correlated with body weight and length at birth {Shields, 2011, 3421491} and cases of intrauterine growth restriction and small-for-gestational-age fetuses are associated with reduced thyroid hormone levels in both human populations and experimental animals {Forhead, 2014, 2344788;Pererira, 2003, 3421496}. Thyroidectomy in fetal sheep reduces total body and organ weights and affects bone development, including delayed maturation and altered bone strength and mineral density {Lanham, 2011, 3421481;Forhead, 2014, 2344788}; these effects were ameliorated by T4 replacement {Forhead, 2014, 2344788}. Furthermore, human congenital hypothyroidism is also associated with neurological and skeletal abnormalities, even when birth weight is unaffected {Patel, 2011, 3421490;Shields, 2011, 3421491}. Based on the broader developmental literature, it is plausible that developmental effects observed following HBCD exposure could be a consequence of HBCD-induced changes in thyroid homeostasis; however, HBCD-specific data to support this relationship are not available.

1.5. Nervous System Effects

1.3.4

1.5.1. Human Evidence

Epidemiology studies have been conducted in children participating in birth cohort studies in the Netherlands {Roze, 2009, 758049} and in adolescents in a cross-sectional general population study in areas around industrial sites in Belgium {Kiciński, 2012, 1927571} ([REF _Ref532818171 \h * MERGEFORMAT]). In a study of children ages 5–6 years (n = 62), maternal HBCD levels measured at week 35 of pregnancy were associated with increased scores for three neuropsychological domains (coordination, total intelligence, and verbal intelligence) after adjusting for maternal education, home environment (Home Observation for Measurement of the Environment [HOME] score), and sex {Roze, 2009, 758049}. The authors stated that no associations were observed between HBCD and the other tested domains (visual perception, visuomotor integration, inhibitory control, attention, behavior, and attention deficit/hyperactivity disorder), but did not report effect estimates for these measures. {Kiciński, 2012, 1927571@@author-year} did not observe associations between HBCD levels and six neurobehavioral measures assessing attention, visual scanning and information processing, working memory, and motor function in a study in adolescents (ages 13–17; n = 515); this analysis was based on HBCD exposure dichotomized at concentrations above and below the LOQ (30 ng/L) because 75% of values were less than the LOQ. Interpretation of the results of these studies is limited by inadequate reporting of results and small sample size in the study by {Roze, 2009, 758049@@author-year}, and by

low HBCD detection rates (<25%) in the study population and measure of HBCD in adolescents that does not represent a relevant time window of exposure for neurodevelopmental outcomes in the case of {Kiciński, 2012, 1927571@@author-year}. Thus, the human evidence is considered inadequate to draw conclusions regarding the relationship between HBCD exposure and nervous system effects.

1.5.2. Animal Evidence

The potential for HBCD to affect the nervous system has been examined in 10 studies in rats {van der Ven, 2006, 787745;Ema, 2008, 787657;WIL Research, 1997, 787758;WIL Research, 2001, 787787;Miller-Rhodes, 2014, 2528337;Eriksson, 2006, 787660;Lilienthal, 2009, 787693;van der Ven, 2009, 589273;Saegusa, 2009, 787721;Genskow, 2015, 2919804} with exposures ranging from a single gavage dose on PND 10 to continuous exposure across two generations.

Discussion of nervous system-related effects is organized by the timing of exposure (i.e., developmental and adult) due to the sensitivity of the developing nervous system to the effect of chemicals. A summary of the evidence pertaining to nervous system effects in experimental animals is presented in [REF _Ref532818186 \h * MERGEFORMAT] and [REF _Ref532818236 \h * MERGEFORMAT]. Individual studies are ordered by study duration and then species. If not otherwise indicated measurements were made in adults.

1.3.4.1 Developmental exposure

Neurodevelopmental milestones

Neurodevelopmental milestones were evaluated in two rat studies {Ema, 2008, 787657;Miller-Rhodes, 2014, 2528337}. Gestational exposure to HBCD heightened tail pinch responses in pooled male and female rat pups (PNDs 1–21; 3–30 mg/kg-day) and reduced forelimb grip strength in juvenile male, but not female, rats (PND 26; 10 and 30 mg/kg-day) {Miller-Rhodes, 2014, 2528337}. Development of sensorimotor reflexes was affected in rats exposed to approximately 1,300 mg/kg-day in a two-generation reproductive toxicity study; however, effects were not consistent across generations, sex, or the reflex evaluated {Ema, 2008, 787657} and were not observed in a separate study {Miller-Rhodes, 2014, 2528337}. Differences in the experimental design (i.e., multigenerational versus developmental) and outcome recording (i.e., righting latency versus age at which ≥85% of pups completed the behavior within 1 minute) may have contributed to differences in the surface righting reflex responses reported by these research groups. Furthermore, in the study by {Ema, 2008, 787657@@author-year}, statistically significant effects on righting reflexes were only observed in exposure groups that also exhibited signs of overt toxicity (e.g., decreased body weight gain and pup survival); thus, changes in sensorimotor reflexes may be due to general toxicity rather than an organ system-specific effect.

Executive function and locomotor activity

The effects of HBCD exposure on executive function (e.g., learning, memory, attention) were evaluated in three studies in rats {Ema, 2008, 787657;Miller-Rhodes, 2014, 2528337} and mice {Eriksson, 2006, 787660}. {Miller-Rhodes, 2014, 2528337@@author-year} evaluated performance on two operant tasks designed to measure sustained attention, response inhibition, and persistence in adult

(11–14 months) and aging rats (19–21 months) that were exposed to HBCD in utero. The go/no-go task evaluated effects on sustained attention and response inhibition by requiring animals to discriminate between distinct visual cues that indicate whether a trial is reinforced for pressing the lever (i.e., go trial) or for abstaining from lever pressing (i.e., no-go trial). Combined responses from male and female offspring from the low-dose group (3 mg/kg-day) showed a statistically significant decrease in the number of correct lever presses and an increase in response latency; however, no effect was observed in the two higher dose groups. No treatment-related effects were observed in the random ratio task, which evaluated persistence behaviors by providing animals with intermittent reinforcement (i.e., food pellet reward) for lever pressing. Although these tests are sensitive indicators of altered cognitive function, the results are difficult to interpret as data were pooled across age cohorts. Furthermore, some aging animals in the 3 mg/kg-day group developed unexplained loss of hindlimb control that was not observed in controls or higher dose groups. To minimize the potential effects on these behavioral outcomes, litters containing animals that developed serious health complications were excluded from analysis {Miller-Rhodes, 2014, 2528337}; however, it is possible that animals with less severe muscular degeneration were included.

Two studies evaluated learning ability using swim maze tests. A statistically significant increase in trial time on a Morris swim maze was observed in young adult (3-month-old) male mice exposed once to 13.5 mg/kg on PND 10; however, swim speed and visual acuity were not measured as possible confounders {Eriksson, 2006, 787660}. In contrast, a statistically significant decrease in trial times on a multiple T-maze was reported on a single day of testing in juvenile F1 male rats (PNW 6) exposed to approximately 100–1,300 mg/kg-day {Ema, 2008, 787657}. Females showed a similar pattern of behavior across multiple testing days, but changes were not statistically significant and the data showed high standard errors (SEs). Differences in the test species, exposure, and testing methods may have contributed to the different results of the two swim maze studies and complicates interpretation of these findings.

Three studies measured effects of early-life exposure on locomotor activity in rats {Ema, 2008, 787657;Miller-Rhodes, 2014, 2528337} and mice {Eriksson, 2006, 787660}. {Eriksson, 2006, 787660@@author-year} evaluated effects in young adult (3-month-old) mice that were administered a single dose on PND 10, which corresponds with a period of rapid growth and maturation for motor and sensory neural networks in mice. Controls and mice exposed to 0.9 mg/kg showed a normal activity pattern, characterized by high initial activity that steadily decreased over the course of the 60-minute test period. The 13.5 mg/kg group, however, exhibited a moderate activity level that remained steady (i.e., significantly lower versus control activity at the beginning and significantly higher versus controls at the end of the test), suggesting failure to habituate to the novel environment of the testing arena. Similar testing methods were employed to evaluate locomotor activity in juvenile {Ema, 2008, 787657}, young adult, and aging rats {Miller-Rhodes, 2014, 2528337}. Although both of these studies utilized longer exposure durations and higher doses, they found no effects on spontaneous locomotor activity {Ema, 2008, 787657;Miller-Rhodes, 2014, 2528337}.

Other neurological effects

Effects on auditory function and dopamine-dependent movement behavior were evaluated in a single rat study that exposed animals continuously throughout gestation, lactation, and into adulthood {Lilienthal, 2009, 787693}. Brainstem evoked auditory potentials (BAEPs) were measured to evaluate effects on auditory function. Study authors reported that males, but not females, showed a small dose-related trend towards increased thresholds and signal latency, suggesting reduced hearing sensitivity. In the same study, dopamine system effects were evaluated by measuring cataleptic movement latencies. Catalepsy is a condition characterized by muscle rigidity and waxy flexibility (i.e., subject tends to remain in a fixed position, but the posture/limb position can be altered). A cataleptic state was induced by haloperidol, a drug that blocks dopamine receptors. Animals were then placed in fixed postures and movement latency was recorded. Statistically significant dose-dependent decreases in movement latency were reported in the catalepsy tests for both sexes, although effects were more pronounced in females. These results suggest that HBCD increases dopamine signaling. It was unclear, however, whether animals were given a recovery period between certain postures in the catalepsy tests, which may have stressed the animals and affected the results. In the BAEP test, the average increase in auditory threshold observed at the highest dose was 9 dB. Although BAEP is a sensitive measure of auditory function, the changes observed in this study were below those generally considered to be biologically significant (10–15 dB).

Three studies evaluated brain weight changes in rats {Ema, 2008, 787657;van der Ven, 2009, 589273;Saegusa, 2009, 787721}. Absolute brain weights showed a statistically significant reduction in F1 adults and both F1 and F2 weanlings in the high-dose group (approximately 1,300 mg/kg-day) {Ema, 2008, 787657}; these animals also exhibited signs of overt toxicity, including decreased viability and pup weight (Section 1.2.4). {van der Ven, 2009, 589273@@author-year} also reported a significant trend for absolute brain weights in male rats at the end of a one-generation exposure, with most groups showing an increase relative to controls; brain weight changes were not observed in females. No statistically significant change in relative brain weight was observed in gestationally and lactationally exposed rats {Saegusa, 2009, 787721}; however, relative brain weight changes are considered to be less informative of nervous system effects. Notably, brain weight changes are considered to be a relatively insensitive measure of neurotoxicity and, with the exception of the F2 high dose animals in {Ema, 2008, 787657@@author-year}, the statistically significant effects were below the level that is considered to be biologically significant.

1.3.4.2 Adult exposure

The four studies that evaluated neurotoxicity endpoints in adult animals did not provide evidence that HBCD exposure affects the nervous system at this life stage {Genskow, 2015, 2919804;WIL Research, 2001, 787787;WIL Research, 1997, 787758;van der Ven, 2006, 787745}. No gross changes in striatal levels of dopamine or its metabolites were observed in adult male mice exposed to 25 mg/kg-day HBCD for 30 days {Genskow, 2015, 2919804}. Similarly, no effects on other neurological measures, including a functional observational battery (FOB), locomotor activity, brain weight, or gross pathology

were observed in adult rats exposed to up to 1,000 mg/kg-day HBCD for 90 {WIL Research, 2001, 787787} or 28 days {WIL Research, 1997, 787758;van der Ven, 2006, 787745}.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Evidence pertaining to nervous system effects in humans

Reference and study design	Results
Studies in infants and children, neurodevelopment	
{Roze, 2009, 758049@@author-year} (the Netherlands, COMPARE cohort, 2001–2002 at baseline) **Population:** Birth cohort, 90 singleton, term births, 62 of 69 (90%) mother-child pairs randomly selected from the cohort for HBCD measures in serum; children ages 5–6 years at follow-up **Exposure measures:** Prenatal exposure, maternal serum at 35th week of pregnancy; 1,2,5,6,9,10-HBCD (HBCD) detected in all samples; LOD 0.8 pg/g serum Median 0.8 (range: 0.3–7.5) ng/g lipids **Effect measures:** Neuropsychological tests (references for procedure provided) • Movement ABC test battery for motor performance (coordination, fine motor skills) • Developmental Coordination Disorder Questionnaire for behavior • Wechsler Preschool and Primary Scale of Intelligence, Revised for intelligence (total, verbal, performance) • Neuropsychological Assessment (NEPSY-II) for visual perception, visuomotor integration, inhibitory control • Rey's Auditory Verbal Learning test (verbal memory) • Test of Everyday Attention for Children (attention) Behavioral tests (references for procedure provided) • Child Behavior Checklist and Teacher's Report Form • Attention Deficit/Hyperactivity Disorder questionnaire **Analysis:** Pearson correlation (for normally distributed variables) or Spearman's rank correlation (for non-normally distributed variables) **Study evaluation[a]:** [EMBED PBrush] Limited analysis and inadequate reporting of results; small sample size	Correlations between lipid-adjusted HBCD and outcome measure adjusted for socioeconomic status (maternal education), HOME score, and sex *Neuropsychological measure* — *Correlation coefficient* Coordination — 0.290 ($p < 0.05$) Total intelligence — 0.393 ($p < 0.05$) Verbal intelligence — 0.479 ($p < 0.01$) (Correlations of similar, but somewhat smaller, magnitude were seen between PCB-153 or 4,4-DDE and coordination; none of the other nine compounds examined were associated with either intelligence measure.) Results for correlations between HBCD and other neuropsychological and behavioral outcomes were not shown, but were stated to be not statistically significant ($p > 0.10$).
Studies in adolescents, neurodevelopment	
Kiciński, 2012, 1927571@@author-year} (Belgium, 2008–2011) **Population:** 515 adolescents (13–17 yrs old) residing in two industrial areas and randomly selected from the general population; participation rates 22–34% in the	Beta (95% CI)[b] Continuous Performance reaction time (msec) (n = 489) — −3.53 (−18.72, 11.67) Continuous Performance — 27.8 (−17.5, 97.9)

three groups; sample size varied by test (designed as "biomonitoring program for environmental health surveillance") **Exposure measures:** Serum samples, HBCD >75% were less than the LOQ (LOQ = 30 ng/L); Median <30 ng/L (range: <LOQ–234) ng/L **Effect measures:** Neurobehavior (Neurobehavioral Evaluation System, NES-3), computerized battery (references for procedure provided) Continuous Performance test (attention) Digit-Symbol test (visual scanning and information processing) Digit Span test (working memory) Finger Tapping (motor function) **Analysis:** Regression models (linear or negative binomial depending on outcome) **Study evaluation[a]:** [EMBED PBrush] Exposure measure does not adequately represent relevant time window of exposure for neuro-developmental outcomes; 75% of HBCD less than the LOD (dichotomized analysis)	errors of omission (%) (n = 489)	
	Continuous Performance errors of commission (%) (n = 489)	21.8 (−2.5, 52.2)
	Digit Symbol total latency (sec) (n = 340)	−0.44 (−6.59, 5.72)
	Digit Span, Forward (n = 511)	0.13 (−0.22, 0.49)
	Digit Span, Backward (n = 499)	−0.04 (−0.39, 0.31)
	Linear regression models for all outcomes except Continuous Performance errors of omission and commission, where negative binomial models were used. All models adjusted for age, gender, type of education, blood lipids, smoking, parental smoking, parental education, and parental home ownership. Additional covariates evaluated included BMI, physical activity, computer use, alcohol and fish consumption, blood lead, and blood PCBs, and were included based on a stepwise regression procedure. Effects of levels above the LOQ were estimated. Models evaluating number of digits in Digital Span test were also adjusted for the method of test administration.	

[a]Evaluation of sources of bias or study limitations (see Systematic Review Methods/Epidemiology Studies, and Appendix B, Table B-3): P = population selection; E = exposure misclassification; O = outcome misclassification; C = confounding; A = analysis; Oth = other feature affecting interpretation of results. Extent of column shading reflects degree of limitation.
[b]Beta is for HBCD >30 ng/L (LOQ) versus <30 ng/L; 0.0 = no association.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Evidence pertaining to neurological effects in animals following developmental exposure to HBCD

Reference and study design	Results			
Neurodevelopmental milestones				
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning through necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/ lactation	**Doses** (mg/kg-d)			
	F1 offspring[a] **0**	**17**	**168**	**1,570**
	F2 offspring[a] **0**	**15**	**139**	**1,360**
	Surface righting reflex response time (s)			
	Male, F1, PND 5 (n = 17–24 litters)			
	Mean (SD) 2.3 (1.1)	2 (0.6)	1.8 (0.5)	1.6* (0.3)
	% of control[b] –	−13%	−22%	−30%
	Female, F1, PND 5 (n = 17–23 litters)			
	Mean (SD) 3.1 (1.8)	2.4 (1.5)	2.9 (2.6)	2.6 (2.6)
	% of control[b] –	−23%	−6%	−16%
	Male, F2, PND 5 (n = 19–22 litters)			

Reference and study design	Results				
	Mean (SD)	2.1 (1.7)	2.0 (1.5)	2.8 (2.5)	2.2 (2.3)
	% of control[b]	–	−5%	33%	5%
	Female, F2, PND 5 (n = 16–22 litters)				
	Mean (SD)	2.3 (0.9)	2.4 (1.7)	2.1 (0.9)	3.7 (3.7)
	% of control[b]	–	4%	−9%	61%
	Mid-air righting reflex completion rate (%)				
	Male, F1, PND 18 (n = 17–23 litters)				
	Mean	100	100	100	100
	% of control[b]	–	0%	0%	0%
	Female, F1, PND 18 (n = 17–23 litters)				
	Mean	100	100	100	100
	% of control[b]	–	0%	0%	0%
	Male, F2, PND 18 (n = 13–22 litters)				
	Mean	100	100	94.4	100
	% of control[b]	–	0%	−6%	0%
	Female, F2, PND 18 (n = 13–21 litters)				
	Mean	100	100	90	76.9*
	% of control[b]	–	0%	−10%	−23%
{Miller-Rhodes, 2014, 2528337@@author-year} Rats, Long-Evans Gavage F1: Continuous maternal exposure throughout gestation	**Doses** (mg/kg-d)				
		0	**3**	**10**	**30**
	Age at which 85% of pups could perform righting reflex				
	Male, F1 (n = 8–10 litters)				
	PND	5	5	5	3
	% of control[b]	–	0%	0%	−40%
	Female, F1 (n = 8–10 litters)				
	PND	7	5	5	3
	% of control[b]	–	−29%	−29%	−57%
	FOB including the righting reflex was conducted every other day from PND 1 to 21. Every pup in each litter was examined.				
	Animals that did not respond to tail pinch (mean % pups per litter)				
	Males and females, F1 PNDs 1–21 (n = 8–10 litters)				
	Mean (SE)	39 (2)	28* (2)	31* (2)	27* (2)
	% of control[b]	–	−28%	−21%	−31%
	Grip strength (Newtons)				
	Male, F1, PND 26 (n = 8–10 litters)				
	Mean (SE)	4.1 (0.2)	3.9 (0.2)	2.8* (0.2)	3.3* (0.2)
	% of control[b]	–	−5%	−32%	−20%
	Data for tail pinch and grip strength were digitized from figure. No significant treatment-related effect on grip strength in females.				
Executive function and locomotor activity					
{Ema, 2008, 787657@@author-year}	**Doses** (mg/kg-d)				
	Male, F1	**0**	**11**	**115**	**1,142**

Reference and study design	Results			
Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/ lactation	**Female, F1**	**0**	**14**	**138**	**1,363**
	Locomotor activity				
	Male, F1, PNW 4 (n = 10)				
		Mean (SD) % of control[b]			
	0–10 min	141.9 (63.5)	240.9 (116.7)	127.4 (79.2)	162.4 (124.9)
		–	70%	−10%	14%
	10–20 min	86.1 (59.3)	116.8 (86.3)	71.7 (44.4)	53.3 (53.7)
		–	36%	−17%	−38%
	20–30 min	39.9 (49.4)	58.2 (66.8)	11.8 (11.4)	8.8 (13.9)
		–	46%	−70%	−78%
	30–40 min	15.6 (19.1)	29.5 (45.0)	2.9 (5.9)	7.1 (11.9)
		–	89%	−81%	−54%
	40–50 min	13.8 (21.5)	5.7 (18.0)	0.0 (0.0)	1.0 (2.5)
		–	−59%	−100%	−93%
	50–60 min	4.8 (15.2)	0.8 (2.5)	0.0 (0.0)	5.7 (18.0)
		–	−83%	−100%	19%
	Female, F1, PNW 4 (n = 10)				
		Mean (SD) % of control[b]			
	0–10 min	196.9 (75.8)	194.1 (112.7)	176.7 (93.8)	172.6 (101.9)
		–	−1%	−10%	−12%
	10–20 min	77.6 (50.0)	70.7 (64.3)	84.7 (66.2)	35.2 (31.8)
		–	−9%	9%	−55%
	20–30 min	40.4 (44.7)	52.1 (62.3)	39.5 (49.4)	17.7 (31.2)
		–	29%	−2%	−56%
	30–40 min	13.0 (30.9)	15.4 (42.0)	5.6 (12.3)	15.8 (22.0)
		–	18%	−57%	22%
	40–50 min	5.4 (14.2)	2.3 (7.3)	9.9 (31.3)	3.6 (11.4)
		–	−57%	83%	−33%
	50–60 min	0.8 (1.9)	1.3 (3.5)	4.9 (12.4)	5.0 (11.2)
		–	63%	513%	525%
	T-maze swim test, trial time (s)				
	Male, F1, PNW 6 (n = 10)				
		Mean (SD) % of control[b]			
	Day 1	8.3 (2.5)	8.0 (1.1)	6.9 (1.3)	8.3 (2.5)
		–	−4%	−17%	0%
	Day 2	48.7 (19.1)	43.5 (18.4)	33.2 (12.0)	40.8 (17.4)
		–	−11%	−32%	−16%
	Day 3	38.9 (14.8)	27.8 (8.8)	32.4* (37.3)	18.4* (4.9)

Reference and study design	Results			
	–	−29%	−17%	−53%
	Day 4	27.5 (12.3)	30.4 (12.3)	28.0 (24.7)	19.6 (5.2)
	–	11%	2%	−29%
	Female, F1, PNW 6 (n = 10)			
		Mean (SD) % of control[b]		
	Day 1	12.2 (4.7)	10.8 (4.0)	8.8 (4.4)	10.5 (2.3)
	–	−11%	−28%	−14%
	Day 2	49.1 (18.2)	43.4 (17.1)	40.7 (14.2)	39.2 (12.2)
	–	−12%	−17%	−20%
	Day 3	42.1 (32.6)	35.1 (15.8)	34.5 (23.3)	31.5 (19.4)
	–	−17%	−18%	−25%
	Day 4	28.3 (8.1)	31.6 (19.6)	30.7 (13.0)	25.4 (10.1)
	–	12%	8%	−10%
{Miller-Rhodes, 2014, 2528337@@author-year} Rats, Long-Evans Gavage F1: Continuous maternal exposure throughout gestation Go/no-go task: animals tested on PNM 14 and 21 RR task animals tested on PNM 11 and 19	**Doses** (mg/kg-d)			
	0	**3**	**10**	**30**
	Go/no-go task (% hits)			
	Males and females, F1 (n = 4)			
	Mean (SE)	94.8 (0.7)	87.8 (1.9)*	94.1 (1.6)	94.8 (0.9)
	% of control[b]	–	−7%	−1%	0%
	Random ratio (RR) task (responses per minute)			
	Males and females, F1 (n = 4)			
		Mean (SD) % of control[b]		
	RR1	8.6 (1.5)	7.5 (0.1)	7.6 (1.2)	8.5 (1.2)
	–	−13%	−12%	−1%
	RR2	14.1 (2.6)	12.8 (1.8)	12.5 (1.5)	14.9 (1.7)
	–	−9%	−11%	6%
	RR5	20.1 (4.0)	20.2 (2.8)	18.9 (2.9)	22.7 (1.5)
	–	1%	−6%	13%
	RR10	26.9 (3.7)	26.4 (4.0)	23.0 (3.6)	25.9 (3.2)
	–	−2%	−15%	−4%
	RR20	24.7 (4.5)	26.5 (3.7)	23.6 (5.3)	30.6 (2.9)
	–	7%	−4%	24%
	All data were digitized from figure. Go/no-go task: hit defined as lever press behavior during a "go" trial. RR task: Different schedules (e.g., RR1, RR2…) correspond to the average number of lever presses between reinforcements.			
{Eriksson, 2006, 787660@@author-year}	**Doses** (mg/kg)			
	0		**0.9**	**13.5**

Reference and study design	Results		
Mice, NMRI Gavage F1: single dose on PND 10 Males only	**Horizontal locomotion** (beam hits)		
	Male, F1, PNM 3 (n = 10)		
		Mean (SD) % of control[b]	
0–20 min	499 (81)	414* (50)	213* (58)
	–	−17%	−57%
20–40 min	209 (62)	256 (50)	232 (39)
	–	22%	11%
40–60 min	12 (8)	12 (16)	256* (47)
	–	0%	2,103%
	Rearing (beam hits)		
	Male, F1, PNM 3 (n = 10)		
		Mean (SD) % of control[b]	
0–20 min	1,596 (285)	1,206* (260)	322*(78)
	–	−24%	−80%
20–40 min	487 (91)	525 (143)	485 (130)
	–	8%	0%
40–60 min	104 (13)	142 (13)	480* (104)
	–	37%	362%
	Total activity (beam hits)		
	Male, F1, PNM 3 (n = 10)		
		Mean (SD) % of control[b]	
0–20 min	4,741 (606)	4,491 (535)	2,495* (321)
	–	−5%	−47%
20–40 min	2,210 (428)	2,424 (606)	2,566 (321)
	–	10%	16%
40–60 min	1,176 (214)	998 (214)	2,709* (570)
	–	−15%	130%
	Morris water maze (s)		
	Male, F1, PNM 3 (n = 12–17)[c]		
		Mean % of control[b]	
Day 1	27	27	25
	–	0%	−1%
Day 2	20	21	23
	–	8%	18%
Day 3	15	17	19
	–	13%	24%
Day 4	10	14*	20*
	–	33%	90%
Day 5	14	20	21*

Reference and study design	Results							
	–			46%			54%	
	All data were digitized from figure. Morris water maze: error data not shown. Day 5, platform relocated.							
Other neurological effects								
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/ lactation	**Doses** (mg/kg-d)							
	F1 offspring[a]	**0**		**17**		**168**		**1,570**
	Male, F1	**0**		**11**		**115**		**1,142**
	Female, F1	**0**		**14**		**138**		**1,363**
	F2 offspring[a]	**0**		**15**		**139**		**1,360**
	Absolute brain weight (mg)							
	Male, F1 PND 26 (n = 17–23)							
	Mean (SD)	1.64 (0.09)		1.66 (0.05)		1.62 (0.07)		1.55* (0.06)
	% of control[b]	–		1%		−1%		−5%
	Female, F1 PND 26 (n = 14–23)							
	Mean (SD)	1.58 (0.09)		1.61 (0.07)		1.59 (0.08)		1.51* (0.06)
	% of control[b]	–		2%		1%		−4%
	Male, F1 adult (n = 22–24)							
	Mean (SD)	2.18 (0.08)		2.22 (0.08)		2.18 (0.09)		2.11* (0.07)
	% of control[b]	–		2%		0%		−3%
	Female, F1 adult (n = 13–22)							
	Mean (SD)	2.07 (0.09)		2.06 (0.07)		2.06 (0.08)		1.97* (0.06)
	% of control[b]	–		0%		0%		−5%
	Male, F2 PND 26 (n = 13–22)							
	Mean (SD)	1.62 (0.13)		1.65 (0.08)		1.60 (0.10)		1.46* (0.09)
	% of control[b]	–		2%		−1%		−10%
	Female, F2 PND 26 (n = 13–22)							
	Mean (SD)	1.57 (0.11)		1.58 (0.07)		1.55 (0.12)		1.41* (0.15)
	% of control[b]	–		1%		−1%		−10%
{Lilienthal, 2009, 787693@@author-year} Rats, Wistar Diet F0: exposure started 10 wks (male) or 2 wks (female) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning until	**Doses** (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	BAEPs, click threshold (dB)								
	Male, F1, PNW 20 (n = 4–6)**								
	Mean (SE)	47 (2)	47 (4)	40 (2)	49 (7)	48 (8)	48 (4)	53 (3)	56 (4)
	% of control[b]	–	0%	−15%	4%	2%	2%	13%	19%
	Female, F1, PNW 20 (n = 4–6)								
	Mean	44 (3)	47 (2)	53 (4)	52 (3)	41 (3)	54 (2)	49 (2)	48 (2)

Reference and study design	Results							
sacrifice (~PNW 20)	(SE)							
	% of control[b]	–	7%	20%	18%	−7%	23%	11%	9%
	Data for males were digitized from figure.							
	Catalepsy, box, foreleg latency (s)							
	Male, F1, PNW 15 (n = 5)**							
	Mean (SE)	135 (24)	150 (18)	105 (19)	98 (26)	129 (27)	140 (27)	99 (33)	69 (30)
	% of control[b]	–	11%	−22%	−27%	−4%	4%	−27%	−49%
	Female, F1, PNW 15 (n = 5)**							
	Mean (SE)	136 (24)	77 (28)	128 (32)	145 (34)	111 (31)	65 (38)	56 (25)	60 (30)
	% of control[b]	–	−43%	−6%	7%	−18%	−52%	−59%	−56%
	Data for females were digitized from figure.							
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F0: exposure started one spermatogenic cycle (males: 70 d) or two estrous cycles (females: 14 d) prior to mating F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Absolute brain weight (g)								
	Male, F1, PNW 11 (n = 4–5)**								
	Mean (SE)	1.84 (0.12)	1.87 (0.07)	1.94 (0.06)	1.98 (0.07)	1.91 (0.07)	1.88 (0.05)	1.92 (0.06)	1.78 (0.06)
	% of control[b]	–	2%	5%	8%	4%	2%	4%	−3%
	Female, F1, PNW 11 (n = 4–5)								
	Mean (SE)	1.76 (0.14)	1.71 (0.09)	1.71 (0.09)	1.77 (0.08)	1.62 (0.23)	1.80 (0.06)	1.76 (0.08)	1.66 (0.07)
	% of control[b]	–	−3%	−3%	1%	−8%	2%	0%	−6%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]F1 and F2 offspring doses presented as mean maternal gestational F0 and F1 doses, respectively.
[b]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[c]Exact number of animals examined per dose group was unclear based on the published paper.

PNM = postnatal month

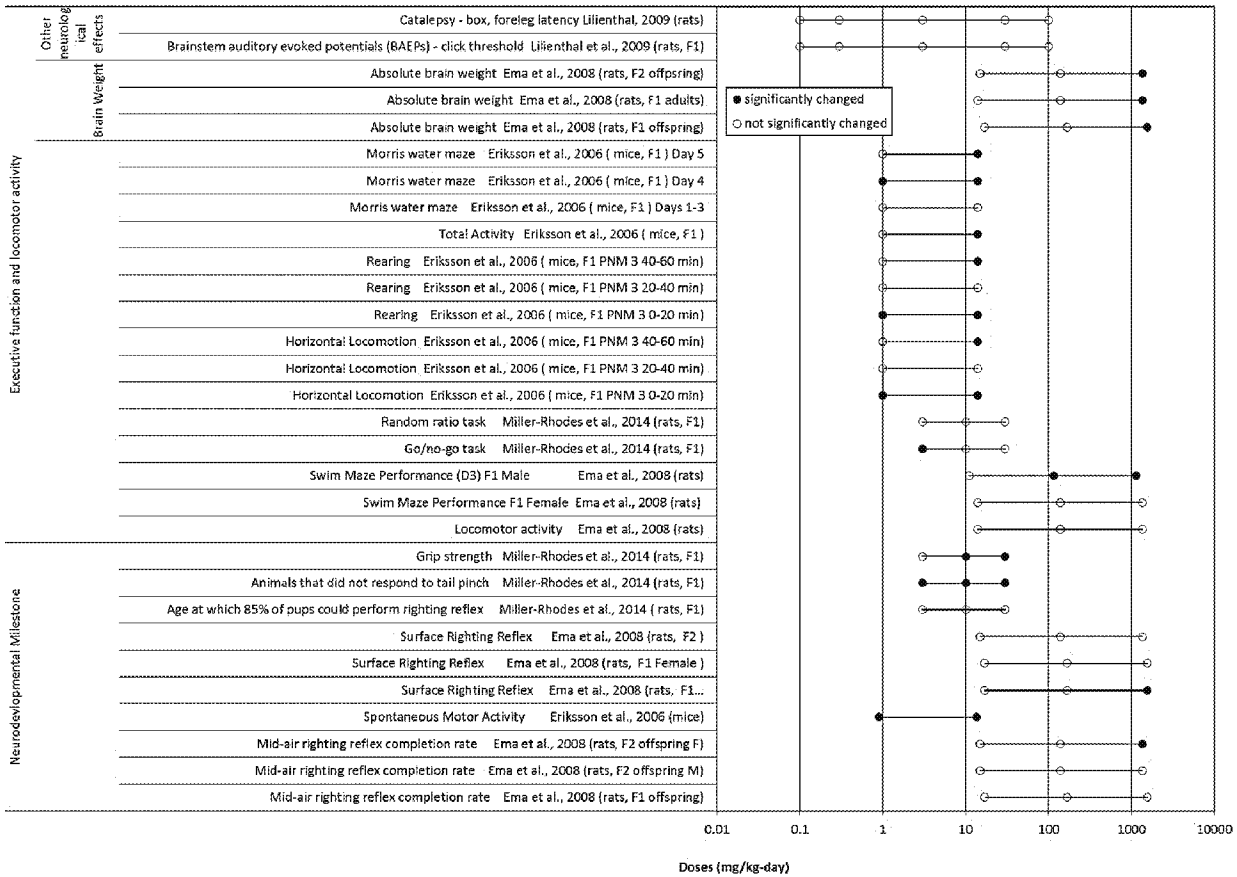

Executive function and locomotor activity
Neurodevelopmental Milestone
Other neurological effects
Brain Weight
Catalepsy - box, foreleg latency Lilienthal, 2009 (rats)
Brainstem auditory evoked potentials (BAEPs) - click threshold Lilienthal et al., 2009 (rats, F1)
Absolute brain weight Ema et al., 2008 (rats, F2 offpsring)
Absolute brain weight Ema et al., 2008 (rats, F1 adults)
Absolute brain weight Ema et al., 2008 (rats, F1 offspring)
Morris water maze Eriksson et al., 2006 (mice, F1) Day 5
Morris water maze Eriksson et al., 2006 (mice, F1) Day 4
Morris water maze Eriksson et al., 2006 (mice, F1) Days 1-3
Total Activity Eriksson et al., 2006 (mice, F1)
Rearing Eriksson et al., 2006 (mice, F1 PNM 3 40-60 min)
Rearing Eriksson et al., 2006 (mice, F1 PNM 3 20-40 min)
Rearing Eriksson et al., 2006 (mice, F1 PNM 3 0-20 min)
Horizontal Locomotion Eriksson et al., 2006 (mice, F1 PNM 3 40-60 min)
Horizontal Locomotion Eriksson et al., 2006 (mice, F1 PNM 3 20-40 min)
Horizontal Locomotion Eriksson et al., 2006 (mice, F1 PNM 3 0-20 min)
Random ratio task Miller-Rhodes et al., 2014 (rats, F1)
Go/no-go task Miller-Rhodes et al., 2014 (rats, F1)
Swim Maze Performance (D3) F1 Male Ema et al., 2008 (rats)
Swim Maze Performance F1 Female Ema et al., 2008 (rats)
Locomotor activity Ema et al., 2008 (rats)
Grip strength Miller-Rhodes et al., 2014 (rats, F1)
Animals that did not respond to tail pinch Miller-Rhodes et al., 2014 (rats, F1)
Age at which 85% of pups could perform righting reflex Miller-Rhodes et al., 2014 (rats, F1)
Surface Righting Reflex Ema et al., 2008 (rats, F2)
Surface Righting Reflex Ema et al., 2008 (rats, F1 Female)
Surface Righting Reflex Ema et al., 2008 (rats, F1...
Spontaneous Motor Activity Eriksson et al., 2006 (mice)
Mid-air righting reflex completion rate Ema et al., 2008 (rats, F2 offspring F)
Mid-air righting reflex completion rate Ema et al., 2008 (rats, F2 offspring M)
Mid-air righting reflex completion rate Ema et al., 2008 (rats, F1 offspring)
significantly changed
not significantly changed
0.01
0.1
1
10
100
1000
10000
Doses (mg/kg-day)

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Exposure response array of nervous system effects following oral exposure.

1.5.3. Mechanistic Evidence

1.3.4.3 Thyroid perturbation and neurodifferentiation

Thyroid hormones are known to play a key role in development of the vertebrate central nervous system, and perinatal exposure to thyroid-disrupting chemicals has been shown to have lasting effects on cognitive and behavioral outcomes {Koibuchi, 2000, 3421479;Howdeshell, 2002, 1442722;Gilbert, 2012, 1609642}. The evidence to support mechanisms by which HBCD may affect thyroid hormones is covered elsewhere (Section 1.2.1, Mechanistic Evidence); therefore, the following discussion focuses on the available studies that specifically investigated possible associations between HBCD-mediated thyroid hormone perturbation and neurodevelopmental endpoints {Saegusa, 2012, 1927608;Ibhazehiebo, 2011, 787676;Ibhazehiebo, 2011, 1402779;Fujimoto, 2013, 1927532}.

As discussed in Section 1.2.1, HBCD elicited a decrease in thyroid hormone levels in developmentally exposed rats {Saegusa, 2009, 787721}. In two follow-up studies by the same research group, thyroid perturbation corresponded with several changes in brain morphometry indicative of altered neuronal migration and neurogenesis in the hippocampus, a region that is critical for learning and memory {Saegusa, 2012, 1927608;Fujimoto, 2013, 1927532}. Developmental exposure also elicited a statistically significant increase in the number of astrocytes and oligodendrocytes in the cingulum, an area of the brain involved in regulating behaviors related to emotion and cognitive function {Fujimoto, 2013, 1927532}. These results mirror those previously found following developmental exposure to known anti-thyroid drugs, propylthiouracil and methimazole {Fujimoto, 2012, 3421482}. These data are supported by two studies with primary rat neuronal cell cultures. During normal development, thyroid hormones regulate neurite growth and arborization of cerebellar granule neurons (CGNs) and Purkinje cells. In the cerebellum, these cells generate a highly interconnected dendritic network that is critical for motor control and coordination {Koibuchi, 2000, 3421479;Gilbert, 2012, 1609642}. Primary rat Purkinje cell {Ibhazehiebo, 2011, 1402779} and CGN {Ibhazehiebo, 2011, 787676} cultures co-exposed to thyroid hormone and sub-nanomolar concentrations of α-HBCD showed statistically significant reductions in thyroid hormone-induced neurite growth and arborization. These effects were seen at concentrations several orders of magnitude below those that reduced viability by >50% in rat primary CGNs {Reistad, 2006, 787719} and human neuroblastoma cells {Al-Mousa, 2012, 1927605}, indicating that they were not due to cytotoxicity. HBCD-mediated effects on neurite growth and arborization could be ameliorated by elevated thyroid hormone levels {Ibhazehiebo, 2011, 1402779} or coexposure with brain-derived neurotrophic factor {Ibhazehiebo, 2011, 787676}.

1.3.4.4 Calcium homeostasis

Several studies suggest that HBCD may alter calcium (Ca^{2+}) homeostasis in the brain by affecting three types of calcium transporters: sarco-endoplasmic reticulum Ca^{2+}-dependent ATPase (SERCA) pumps {Al-Mousa, 2014, 2343726;Al-Mousa, 2012, 1927605}, ligand-gated Ca^{2+} channels (LGCC) {Reistad, 2006, 787719}, and voltage-gated Ca^{2+} channels (VGCC) {Dingemans, 2009, 1927726}. Within neurons, Ca^{2+} levels are typically maintained at low concentrations relative to the extracellular fluid; however, rapid influx can occur through various ion channels. After an influx event,

low cytosolic Ca2+ levels are restored via active transport across the cell membrane or sequestration into subcellular compartments. Tight regulation of Ca2+ is critical as both excess and insufficient levels can adversely affect numerous cellular processes.

SERCA uses ATP to actively transport excess Ca2+ from the cytosol into intracellular compartments to regulate protein synthesis and neurotransmitter release {Neher, 2008, 504991;Rodriguez, 2001, 3421501}. HBCD increased intracellular Ca2+ and cell death in human neuroblastoma cells (SH-SY5Y) via concentration-dependent SERCA inhibition {Al-Mousa, 2014, 2343726;Al-Mousa, 2012, 1927605}. HBCD interacts with SERCA in a manner that: (1) reduces ATP binding affinity and (2) stabilizes the low Ca2+ affinity conformation {Al-Mousa, 2014, 2343726}. Exposure of PC12 cells to either the technical mixture or individual HBCD isomers reduced Ca2+ influx through VGCCs, but did not affect resting intracellular Ca2+ levels {Dingemans, 2009, 1927726}. γ-HBCD showed the greatest potency, whereas the α-isomer had a moderate effect similar to that of the technical mixture. These effects were associated with decreased catecholamine release, likely due to low cytosolic Ca2+ levels that were insufficient to trigger synaptic release {Neher, 2008, 504991}. HBCD may also act as a mild LGCC-agonist. Co-exposure to MK801, an LGCC antagonist, was found to ameliorate HBCD-induced cytotoxicity, suggesting a role of this Ca2+ channel in neurotoxicity. Although no significant changes in intracellular Ca2+ calcium were reported, this was the only study that measured Ca2+ effects as an average across all cells, which may have reduced the sensitivity when compared to single cell measurements {Al-Mousa, 2012, 1927605;Dingemans, 2009, 1927726}.

1.3.4.5 Neurotransmitter reuptake

Adult male mice exposed to 25 mg/kg-day for 30 days showed decreased striatal levels of dopamine transporter and vesicular monoamine transporter 2, regulators of dopamine homeostasis and neurotransmission {Genskow, 2015, 2919804}. Similarly, an in vitro study found a dose-related reduction in dopamine and gamma-aminobutyric acid uptake in rat synaptosomes and vesicles exposed to HBCD {Mariussen, 2003, 787695}. Although prolonged deficits in reuptake mechanisms could result in excessive stimulation of the post synaptic cell or deplete neurotransmitter stores in the presynaptic cell, {Genskow, 2015, 2919804@@author-year} did not find significant changes in tissue concentrations of dopamine or its metabolites in adult mice exposed for 30 days.

1.6. Immune System Effects

1.3.5

1.6.1. Human Evidence

The potential for HBCD to affect the immune system has not been investigated in humans.

1.6.2. Animal Evidence

The potential for HBCD to affect the immune system has been examined in eight studies in rats {van der Ven, 2009, 589273;van der Ven, 2006, 787745;Hachisuka, 2010, 2919532;Ema, 2008, 787657;WIL Research, 1997, 787758;WIL Research, 2001, 787787} and mice {Maranghi, 2013, 1927558;Watanabe, 2010, 1927692}, with exposures ranging from a 28-day exposure in adults to continuous exposure across two generations.

Discussion of immune-related effects of HBCD is organized first by age of exposure (i.e., developmental or adult) and second by the type of endpoint evaluated (i.e., functional or observational). Exposure timing is an important factor that may influence the effect of chemical exposure on immune function, particularly for early-life exposure studies. In rodents, immune development occurs in a series of discrete stages until approximately PND 42. The developing immune system is susceptible to perturbation resulting from chemical exposure, and exposures during this period may result in distinct toxicological consequences that would not be observed in animals exposed only as adults {Burns-Naas, 2008, 1011861}. With regard to the type of endpoint evaluated, functional immune outcomes, including response to challenge with an infectious agent or immunization with a foreign antigen, are the most relevant and sensitive for determining potential immunotoxicity because the primary role of the immune system is to protect host integrity from foreign challenge and potential insult. Laboratory animals are housed in environments that limit their exposure to antigenic stimulation or infectious agents, and their immune systems are typically in a resting state {WHO, 2012, 1249755}. In the absence of a foreign challenge, observational endpoints, including structural alterations or changes in immune cell populations, can provide information about immune system effects, but are considered less sensitive and predictive {Luster, 2005, 2174509}.

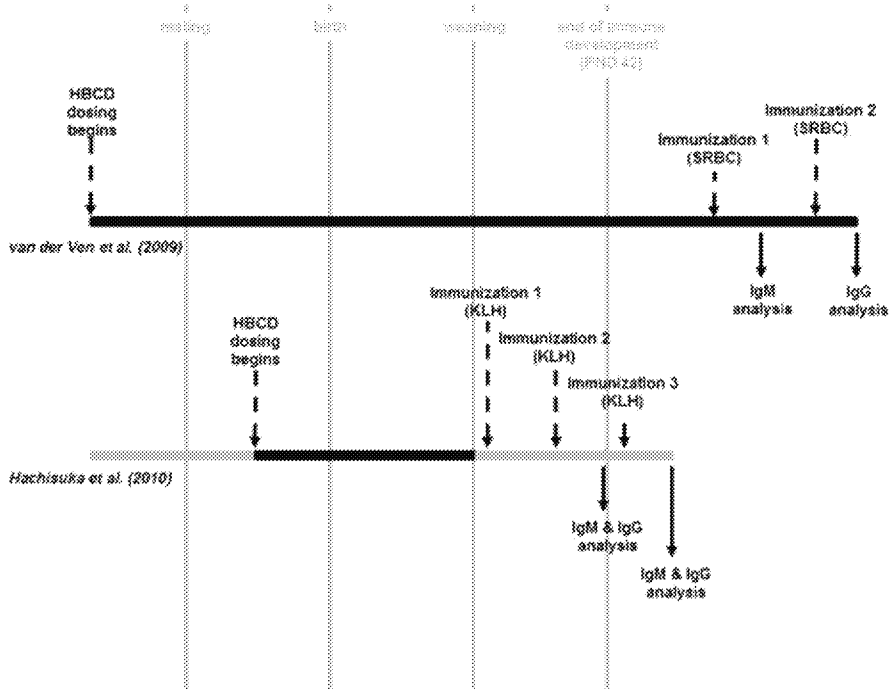
A summary of the evidence pertaining to functional and observational immune system effects in experimental animals is presented in [REF _Ref532818845 \h * MERGEFORMAT], [REF _Ref532818852 \h * MERGEFORMAT], [REF _Ref532818857 \h * MERGEFORMAT] and [REF _Ref532818885 \h * MERGEFORMAT]. Studies are ordered within effect categories by decreasing exposure duration and then species.

1.3.5.1 Developmental exposure

Functional immune effects

Changes in functional immune endpoints (immunoglobulin G [IgG] and immunoglobulin [IgM] antibody production in response to foreign antigens) following developmental HBCD exposures were evaluated in two one-generation reproductive toxicity studies in male {van der Ven, 2009, 589273} or female rats {Hachisuka, 2010, 2919532} (see Table C-3 and Figure C-4). Statistically significant changes in IgG levels were reported in both studies, but with opposite

directions of effect; males exposed to up to 100 mg/kg-day showed a dose-dependent increase in IgG, whereas females exposed to approximately 1,500 mg/kg-day showed a decrease. Differences in the design of these two studies, including timing of exposure, immune challenge, and titer measurement (Figure C-3), may have contributed to the inconsistent results. IgM activity was unaffected in {van der Ven, 2009, 589273@@author-year} and results were not reported by {Hachisuka, 2010, 2919532@@author-year}. {van der Ven, 2009, 589273@@author-year} also evaluated natural killer (NK) cell activity and found no treatment-related effects.



KLH = keyhole limpet hemocyanin; SRBC = sheep red blood cell

Horizontal lines represent the experimental timelines, with black indicating the time period when HBCD was administered (i.e., from 2 weeks prior to mating through IgG analysis in {van der Ven, 2009, 589273@@author-year}, and from GD 10 to PND 21 in {Hachisuka, 2010, 2919532@@author-year}).

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Comparison of study designs used by {van der Ven, 2009, 589273@@author-year} and {Hachisuka, 2010, 2919532@@author-year}.

Observational immune effects

Five studies evaluated effects on observational immune parameters, including organ weights, hematology, and histopathology, in developmentally-exposed rats {Ema, 2008, 787657;van der Ven, 2009, 589273;Hachisuka, 2010, 2919532;Saegusa, 2009, 787721} or mice {Maranghi, 2013, 1927558} (see Table C-4 and Figure C-4).

Thymus weights showed significant dose-response trends in male and female adult rats (PNW 11) continuously exposed to HBCD at doses up to 100 mg/kg-day {van der Ven, 2009, 589273} and in female F2 weanlings exposed to approximately 1,300 mg/kg-day HBCD throughout gestation and lactation {Ema, 2008, 787657}. Spleen weight was reduced in both male and female F2 weanlings from the 1,300 mg/kg-day dose group {Ema, 2008, 787657}. A significant positive trend was also reported for absolute popliteal lymph node weight in PNW 11 male, but not female, rats {van der Ven, 2009, 589273}. No other treatment-related effects were reported for thymus {Hachisuka, 2010, 2919532;Saegusa, 2009, 787721;Maranghi, 2013, 1927558} or spleen weights {Hachisuka, 2010, 2919532;Saegusa, 2009, 787721;Maranghi, 2013, 1927558;van der Ven, 2009, 589273}.

Hematological analyses revealed significant treatment-related effects on several blood immune cell populations, although the pattern of effect was variable across studies, sex, and time point. Total white blood cell (WBC) count was measured in three studies. {Hachisuka, 2010, 2919532@@author-year} reported statistically significant increases in WBC count in HBCD-exposed male rats on PNWs 3 and 11 (approximately 8 weeks after the end of the exposure). In contrast, {van der Ven, 2009, 589273@@author-year} reported a significant dose-related decrease in continuously exposed PNW 11 male rats, and {Ema, 2008, 787657@@author-year} found no effect on total WBCs of F1 males or females. In addition to total WBCs, several subpopulations were measured. {van der Ven, 2009, 589273@@author-year} found a significant dose-related increase and decrease in the fraction of neutrophils and lymphocytes, respectively. The magnitude of the lymphocyte change was small (≤4% change from control) and the biological significance is unclear. {Hachisuka, 2010, 2919532@@author-year} also measured subpopulations of several leukocyte subtypes. On PNW 3, high-dose (1,505 mg/kg-day HBCD) male rats showed a decrease in activated T-cell and NK cell fractions and an increase in inactive B-cell fractions; however, cell fractions returned to control levels by PNW 11.

{Hachisuka, 2010, 2919532@@author-year} and {van der Ven, 2009, 589273@@author-year} reported inconsistent effects on splenic NK and cytotoxic T-cell

populations. {Hachisuka, 2010, 2919532@@author-year} reported a statistically significant decrease in the NK cell fraction (e.g., CD4NKT cells, PNW 3) and an increase in the cytotoxic T-cell fraction in adult rats (CD8+ cells, PNW 11) that were gestationally and lactationally exposed to HBCD. In contrast, male rats continuously exposed through PNW 11 showed a dose-dependent increase in the NK cell fraction and no change in the cytotoxic T-cell fraction. No other treatment-related effects were observed for other immune cell counts in the spleen {van der Ven, 2009, 589273}.

Immune cell counts were also measured in the thymus {Hachisuka, 2010, 2919532} and bone marrow {van der Ven, 2009, 589273}. Rats showed decreases in the thymus fraction of active and regulatory T-cells and an increase in NK cells on PNW 3 and PNW 11, respectively {Hachisuka, 2010, 2919532}. WBC counts in bone marrow showed an increasing dose-related trend in adult males continuously exposed to HBCD at doses up to 100 mg/kg-day {van der Ven, 2009, 589273}.

Histological examination of immune-related tissues showed limited changes with no clear pattern of effect. Thymus tissues showed increased incidence of "starry sky" appearance {Hachisuka, 2010, 2919532} and blurring of the corticomedullary demarcation {Maranghi, 2013, 1927558} in rats and mice, respectively. In the spleen, increased incidence of marginal zone enlargement was also observed in adult (PNW 11) rats continuously exposed to 100 mg/kg-day HBCD {van der Ven, 2009, 589273}. No other treatment-related histological changes were observed {Hachisuka, 2010, 2919532;van der Ven, 2009, 589273;Ema, 2008, 787657}.

1.3.5.2 Adult exposure

Functional immune effects

Two studies evaluated functional immune endpoints following adult exposure to HBCD for 28 days {van der Ven, 2006, 787745;Watanabe, 2010, 1927692}. No statistically significant changes were observed in NK cell activity in adult male rats {van der Ven, 2006, 787745} or host immunity infection in female mice {Watanabe, 2010, 1927692}.

Observational immune effects

Treatment related effects on organ weight, hematology, and histopathology were evaluated in four rat studies {van der Ven, 2006, 787745;Ema, 2008, 787657;WIL Research, 1997, 787758;WIL Research, 2001, 787787} (see Table C-5 and Figure C-4). Trends identified by the authors as statistically significant were reported for absolute thymus weight in male rats and for absolute spleen weight in female rats administered up to 200 mg/kg-day for 28 days {van der Ven, 2006, 787745}. In both cases, effects were not consistent across sexes, the magnitude of the effect was small, and the biological significance of these changes is unclear.

Hematological analyses revealed a statistically significant reduction in the percentage of stabform and segmented neutrophils and increase in the lymphocyte fraction of F0 females exposed to HBCD for 14 weeks {Ema, 2008, 787657}; however, these effects were only seen in the low-dose group (approximately 14 mg/kg-day) in this study and not in a second study involving adult exposure {van der Ven, 2006, 787745}. Total splenocyte number was decreased in adult male rats in the 28-day study by {van der Ven, 2006, 787745@@author-year}. No other observational immune endpoints were affected {Ema, 2008, 787657;WIL Research, 1997, 787758;WIL Research, 2001, 787787}.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Evidence pertaining to functional immune system effects in animals following exposure to HBCD during development

Reference and study design	Results							
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)							
	Male, F1	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	SRBC antibody titers IgG (extinction)								
	Male, F1, PNW 11 (n = 2–4)**								
	Mean (SD)	0.182 (0.128)	0.362 (0.333)	0.174 (0.143)	0.233 (0.169)	0.152 (0.180)	0.444 (0.143)	0.856 (0.231)	0.469 (0.205)
	% change[a]	–	99%	−4%	28%	−16%	144%	370%	158%
	Animals (males only) immunized with SRBCs on PNWs 8 and 10.								
{Hachisuka, 2010, 2919532@@author-year} Rats, SD:IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11	**Doses** (mg/kg-d)[b]							
	Female, F1	**0**		**14.8**		**146.3**		**1,505**	
	Antibody IgG responses to KLH (titer)								
	Female, F1, PND 40 (n = 7–8, estimated from graph)								
	Mean	139,452		63,196		95,592		42,548*	
	% change[a]	–		−55%		−31%		−69%	
	Data were digitized from figure; animals (females only) challenged with KLH on PNDs 23 and 33. IgM titers (enzyme-linked immunosorbent assay) were measured on PND 40.								

*Statistically significantly different from the control at $p < 0.05$.
**Significant dose response trend.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[b]TWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Evidence pertaining to observational immune system effects in animals following exposure to HBCD during development

Reference and study design	Results				
Organ weight					
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d)				
	F1 offspring[a]	**0**	**17**	**168**	**1,570**
	Male, F1	**0**	**11**	**115**	**1,142**
	Female, F1	**0**	**14**	**138**	**1,363**
	F2 offspring[a]	**0**	**15**	**139**	**1,360**
	Absolute spleen weight (mg)				
	Male, F1, adult (n = 22–24)				
	Mean (SD)	885 (168)	840 (147)	878 (163)	851 (113)
	% change[b]	–	−5%	−1%	−4%
	Male, F1, PND 26 (n = 17–23)				
	Mean (SD)	336 (62)	327 (41)	334 (43)	309 (69)
	% change[b]	–	−3%	−1%	−8%
	Female, F1, adult (n = 13–22)				
	Mean (SD)	632 (124)	595 (68)	624 (93)	578 (70)
	% change[b]	–	−6%	−1%	−9%
	Female, F1, PND 26 (n = 14–23)				
	Mean (SD)	311 (53)	306 (44)	304 (59)	280 (40)
	% change[b]	–	−2%	−2%	−10%
	Male, F2, PND 26 (n = 13–22)				
	Mean (SD)	360 (83)	361 (54)	346 (78)	263* (50)
	% change[b]	–	0%	−4%	−27%
	Female F2, PND 26 (n = 13–21)				
	Mean (SD)	325 (59)	302 (42)	299 (62)	225* (45)
	% change[b]	–	−7%	−8%	−31%
	Absolute thymus weight (mg)				
	Male, F1, adult (n = 22–24)				
	Mean (SD)	344 (72)	305 (92)	368 (100)	341 (76)
	% change[b]	–	−11%	7%	−1%
	Female, F1, adult (n = 13–22)				
	Mean (SD)	250 (62)	233 (62)	276 (80)	259 (76)
	% change[b]	–	−7%	10%	4%
	Male, F1, PND 26 (n = 17–23)				
	Mean (SD)	342 (68)	339 (50)	369 (59)	317 (57)
	% change[b]	–	−1%	8%	−7%
	Female, F1, PND 26 (n = 14–23)				
	Mean (SD)	335 (64)	330 (58)	370 (58)	305 (31)
	% change[b]	–	−1%	10%	−9%
	Male, F2, PND 26 (n = 13–22)				

Reference and study design	Results							
	Mean (SD)	343 (92)	336 (57)	360 (88)	282 (71)			
	% change[b]	–	−2%	5%	−18%			
	Female, F2, PND 26 (n = 13–22)							
	Mean (SD)	338 (85)	324 (50)	331 (69)	260* (80)			
	% change[b]	–	−4%	−2%	−23%			
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Doses** (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Absolute popliteal lymph node weight (mg)								
	Male, F1 (n = 4–5)**								
	Mean (SD)	9 (2)	10 (3)	9 (4)	15 (11)	9 (3)	8 (1)	10 (5)	21 (16)
	% change[b]	–	11%	0%	67%	0%	−11%	11%	133%
	Female, F1 (n = 4–5)								
	Mean (SD)	8 (2)	9 (2)	9 (2)	8 (2)	8 (2)	8 (2)	9 (1)	7 (2)
	% change[b]	–	12%	12%	0%	0%	0%	12%	−12%
	Absolute spleen weight (g)								
	Male, F1 (n = 4–5)								
	Mean (SD)	0.49 (0.12)	0.53 (0.07)	0.49 (0.03)	0.58 (0.07)	0.49 (0.05)	0.50 (0.07)	0.58 (0.09)	0.48 (0.06)
	% change[b]	–	8%	0%	18%	0%	2%	18%	−2%
	Female, F1 (n = 4–5)								
	Mean (SD)	0.40 (0.04)	0.39 (0.04)	0.37 (0.06)	0.56 (0.37)	0.56 (0.42)	0.38 (0.05)	0.40 (0.04)	0.39 (0.07)
	% change[b]	–	−3%	−8%	40%	40%	−5%	0%	−3%
	Absolute thymus weight (g)								
	Male, F1 (n = 4–5)**								
	Mean (SD)	0.62 (0.10)	0.54 (0.12)	0.53 (0.12)	0.56 (0.13)	0.50 (0.09)	0.55 (0.08)	0.48 (0.14)	0.45 (0.06)
	% change[b]	–	−13%	−15%	−10%	−19%	−11%	−23%	−27%
	Female, F1 (n = 4–5)**								
	Mean (SD)	0.49 (0.07)	0.41 (0.05)	0.40 (0.04)	0.42 (0.05)	0.48 (0.10)	0.45 (0.06)	0.44 (0.11)	0.37 (0.07)
	% change[b]	–	−16%	−18%	−14%	−2%	−8%	−10%	−24%
{Hachisuka, 2010, 2919532@@author-year} Rats, SD:IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11	**Doses** (mg/kg-d)[c]	**0**	**15**	**146**	**1,505**				
	Absolute spleen weight (g)								
	Male, F1, PNW 3 (n = 10)								
	Mean (SD)	0.29 (0.05)	0.25 (0.03)	0.22 (0.04)	0.23 (0.04)				
	% change[b]	–	−14%	−24%	−21%				
	Male, F1, PNW 11								
	Mean (SD)	0.55 (0.08)	0.55 (0.11)	0.56 (0.08)	0.53 (0.13)				
	% change[b]	–	0%	2%	−4%				
	Absolute thymus weight (g)								
	Male, F1, PNW 3 (n = 10)								

Reference and study design	Results							
Only males evaluated	Mean (SD)	0.21 (0.06)	0.24 (0.05)	0.21 (0.06)	0.21 (0.03)			
	% change[b]	–	14%	0%	0%			
	Male, F1, PNW 11 (n = 10)							
	Mean (SD)	0.79 (0.08)	0.88 (0.17)	0.88 (0.18)	0.81 (0.13)			
	% change[b]	–	11%	11%	3%			
Hematology								
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: maternal exposure throughout gestation/lactation; dietary exposure post weaning until necropsy	**Doses** (mg/kg-d)							
	Male, F1	**0**	**11**	**115**	**1,142**			
	Female, F1	**0**	**14**	**138**	**1,363**			
	Lymphocyte fraction (%)							
	Male, F1 (n = 10)							
	Mean (SD)	88.2 (4.4)	90.9 (2.7)	87.7 (5.9)	87.3 (5.7)			
	% change[b]	–	3%	−1%	−1%			
	Female, F1 (n = 10)							
	Mean (SD)	83.6 (9.4)	76.2 (9.6)	83.6 (8.3)	73 (11.6)			
	% change[b]	–	−9%	0%	−13%			
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11 Only males evaluated	**Doses** (mg/kg-d)	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	Basophil cell count in blood (×10⁹/L)								
	Male, F1 (n = 3–4)**								
	Mean (SD)	0.040 (0.004)	0.072 (0.016)	0.063 (0.026)	0.057 (0.016)	0.045 (0.016)	0.048 (0.028)	0.068 (0.008)	0.035 (0.030)
	% change[b]	–	80%	57%	43%	12%	20%	70%	−12%
	Lymphocyte cell fraction in blood (%)								
	Male, F1 (n = 3–4)**								
	Mean (SD)	89.64 (0.29)	89.87 (0.26)	89.45 (0.29)	89.72 (0.18)	88.61 (0.4)	89.61 (0.25)	88.65 (0.15)	85.9 (0.23)
	% change[b]	–	0%	0%	0%	−1%	0%	−1%	−4%
	WBC count in blood (×10⁹/L)								
	Male, F1 (n = 3–4)**								
	Mean (SD)	5.10 (1.01)	7.18 (1.44)	5.72 (1.79)	6.53 (0.72)	4.90 (1.71)	5.92 (2.27)	6.55 (0.14)	4.05 (1.50)
	% change[b]	–	41%	12%	28%	−4%	16%	28%	−21%
{Hachisuka, 2010, 2919532@@author-year} Rats, SD:IGS Diet F1: maternal	**Doses** (mg/kg-d)[c]	**0**	**14.8**	**146.3**	**1,505**				
	Activated T cell fraction in blood (%)								
	Male, F1, PNW 3 (n = 10)								
	Mean (SD)	13.51 (3.47)	14.01 (2.16)	11.81 (1.96)	10.40* (2.02)				
	% change[b]	–	4%	−13%	−23%				

Reference and study design	Results							
exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11 Only males evaluated	**Male, F1, PNW 11** (n = 10)							
	Mean (SD)	1.45 (0.54)	1.35 (0.6)	1.27 (0.47)	1.32 (0.24)			
	% change[b]	–	−7%	−12%	−9%			
	Lymphocyte fraction in blood (%)							
	Male, F1, PNW 3 (n = 9–10)							
	Mean (SD)	78.88 (4.74)	79.02 (3.18)	81.69 (3.81)	81.41 (4.06)			
	% change[b]	–	0%	3%	3%			
	Male, F1, PNW 11 (n = 10)							
	Mean (SD)	84.64 (5.46)	84.27 (4.88)	87.56 (4.33)	86.44 (3.36)			
	% change[b]	–	0%	3%	2%			
	NK cell fraction in blood (%)							
	Male, F1, PNW 3 (n = 10)							
	Mean (SD)	0.12 (0.03)	0.1 (0.03)	0.09 (0.02)	0.08* (0.04)			
	% change[b]	–	−17%	−25%	−33%			
	Male, F1, PNW 11 (n = 10)							
	Mean (SD)	0.27 (0.07)	0.23 (0.08)	0.27 (0.07)	0.25 (0.09)			
	% change[b]	–	−15%	0%	−7%			
	WBC count in blood ($\times 10^2/\mu L$)							
	Male, F1, PNW 3 (n = 10)							
	Mean (SD)	35.3 (11.3)	30.9 (10)	47.5* (11.8)	39.6 (7.9)			
	% change[b]	–	−12%	35%	12%			
	Male, F1, PNW 11 (n = 10)							
	Mean (SD)	82.1 (17.8)	109.8* (30.8)	110* (29.3)	103.4 (34.1)			
	% change[b]	–	34%	34%	26%			
Histopathology								
{van der Ven, 2009, 589273@@author-year} Rats, Wistar Diet One generation F1: continuous maternal exposure throughout gestation/lactation; dietary exposure post weaning through PNW 11	**Male, F1** **Female, F1**	**0**	**0.1**	**0.3**	**1**	**3**	**10**	**30**	**100**
	WBC count in bone marrow ($\times 10^9$/L)								
	Male, F1 (n = 3–4)**								
	Mean (SD)	9.3 (3.4)	15.0 (9.3)	17.4 (8.5)	13.0 (3.0)	17.9 (4.2)	20.2 (4.1)	16.3 (5.0)	17.6 (4.8)
	% change[b]	–	61%	87%	40%	92%	117%	75%	89%
	CD161a (NK) subpopulation fraction in spleen (%)								
	Male, F1 (n = 3–5)**								
	Mean (SD)	7.9 (0.4)	8.8 (0.8)	8.6 (1.4)	8.9 (1.3)	9.6 (0.6)	8.9 (0.8)	9.0 (1.5)	11.3 (1.3)
	% change[a]	–	11%	9%	13%	22%	13%	14%	43%
	Splenic marginal zone enlargement (incidence)								
	Male, F1 (n = 8–10)								
	Incidence	1/8	–[d]	–[d]	–[d]	–[d]	–[d]	–[d]	7/10*
{Hachisuka, 2010, 2919532@@author-	**Doses** (mg/kg-d)[c]								
	Male, F1	**0**		**15**		**146**		**1,505**	

Reference and study design	Results			
year} Rats, SD:IGS Diet F1: maternal exposure from GD 10 to PND 20 followed by an 8-wk recovery period through PNW 11	**Female, F1**			
	CD4NKT (NK) cell fraction in spleen (%)			
	Male, F1, PNW 3 (n = 10)			
	Mean (SD)	6.47 (0.61)	6.28 (0.81)	6.4 (1.31)	5.63* (0.81)
	% change[b]	–	−4%	−1%	−13%
	Male, F1, PNW 11 (n = 10)			
	Mean (SD)	12.53 (1.88)	12.89 (1.85)	13.78 (2.66)	13.09 (1.72)
	% change[b]	–	3%	10%	4%
	CD8+ CD4- (cytotoxic T-cell) cell fraction in spleen (%)			
	Male, F1, PNW 3 (n = 10)			
	Mean (SD)	6.86 (0.95)	8.12 (2.16)	6.99 (1.42)	6.43 (1.44)
	% change[b]	–	28%	10%	1%
	Male, F1, PNW 11 (n = 10)			
	Mean (SD)	14.42 (2.23)	18.54* (4.34)	16.85 (4.31)	18.87* (4.82)
	% change[b]	–	29%	17%	31%
	N NKRP1A+CD4- (NK) cell fraction in spleen (%)			
	Male, F1, PNW 3 (n = 10)			
	Mean (SD)	5.75 (0.35)	6.06 (1.09)	5.65 (0.87)	5.09* (0.76)
	% change[b]	–	5%	−2%	−11%
	Male, F1, PNW 11 (n = 10)			
	Mean (SD)	10.63 (1.63)	9.97 (3.44)	11.38 (2.47)	9.44 (2.39)
	% change[b]	–	−6%	7%	−11%
	Activated T-cell fraction in thymus (%)			
	Male, F1, PNW 3 (n = 10)			
	Mean (SD)	2.67 (0.87)	2.46 (0.80)	1.82* (0.55)	1.87 (1.15)
	% change[b]	–	−4%	−29%	−27%
	Male, F1, PNW 11 (n = 10)			
	Mean (SD)	0.92 (0.97)	0.74 (0.51)	1.02 (0.84)	1.04 (0.70)
	% change[b]	–	−20%	11%	13%
	Increased starry sky appearance in thymus			
	Male, F1, PNW 3 (n = 10)			
	Incidence	0/10	0/10	4/10*	1/10
	Male, F1, PNW 11 (n = 10)			
	Incidence	0/10	0/10	0/10	0/10
	Female, F1, PNW 3 (n = 10)			
	Incidence	0/10	0/10	0/10	0/10
	Female, F1, PNW 11 (n = 10)			
	Incidence	0/10	0/10	3/10	0/10
	NK cell fraction in thymus (%)			
	Male, F1, PNW 3 (n = 10)			
	Mean (SD)	0.07 (0.03)	0.07 (0.03)	0.06 (0.02)	0.07 (0.05)
	% change[b]	–	0%	−43%	0%

Reference and study design	Results			
	Male, F1, PNW 11 (n = 10)			
	Mean (SD)	0.2 (0.04)	0.2 (0.05)	0.25 (0.09)	0.27* (0.08)
	% change[b]	–	0%	25%	35%
	Treg cell fraction in thymus (%)			
	Male, F1, PNW 3 (n = 10)			
	Mean (SD)	7.7 (2.57)	5.15* (0.94)	7.69 (1.27)	7.85 (2.85)
	% change[b]	–	−33%	0%	−5%
	Male, F1, PNW 11 (n = 10)			
	Mean (SD)	4.16 (1.09)	3.98 (0.87)	4.41 (0.76)	4.32 (1.22)
	% change[b]	–	−1%	6%	4%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100.
[b]F1 and F2 offspring doses presented as mean maternal gestational F0 and F1 doses, respectively.
[c]TWAs for each exposure group were calculated by: (1) multiplying the measured HBCD intake (mg/kg-day) reported by the study authors for GDs 10–20, PNDs 1–9, and PNDs 9–20 by the number of inclusive days of exposure for each time period; (2) adding the resulting products together; and (3) dividing the sum by the total number of inclusive days (33) of HBCD exposure. Example: 100 ppm = (8.1 mg/kg-day × 11 days) + (14.3 mg/kg-day × 10 days) + (21.3 mg/kg-day × 12 days)/33 days = 14.8 mg/kg-day.
[d]Not measured; only control and high-dose values reported.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Evidence pertaining to observational immune system effects in animals following exposure to HBCD as adults

Reference and study design	Results			
Organ weight				
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: dietary exposure post weaning until necropsy F1/F2 offspring: continuous maternal exposure throughout gestation/lactation	**Doses** (mg/kg-d)			
	Male, F0	**0**	**10**	**101**	**1,008**
	Female, F0	**0**	**14**	**141**	**1,363**
	Absolute spleen weight (mg)			
	Male, F0 (n = 22–24)			
	Mean (SD)	848 (136)	828 (109)	855 (160)	843 (248)
	% change[a]	–	−2%	1%	−1%
	Female, F0 (n = 17–24)			
	Mean (SD)	588 (75)	577 (83)	570 (89)	584 (72)
	% change[a]	–	−2%	−3%	−1%
	Absolute thymus weight (mg)			
	Male, F0 (n = 22–24)			
	Mean (SD)	323 (88)	305 (82)	299 (64)	315 (71)
	% change[a]	–	−6%	−7%	−2%
	Female, F0 (n = 17–24)			
	Mean (SD)	232 (38)	238 (63)	252 (73)	200 (64)
	% change[a]	–	3%	9%	−14%

Reference and study design	Results							
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure starting on PNW 11	**Doses** (mg/kg-d)	**0**	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	Absolute spleen weight (g)								
	Male (n = 4–5)								
	Mean (SD)	0.51 (0.09)	0.59 (0.13)	0.78 (0.55)	0.52 (0.05)	0.58 (0.08)	0.47 (0.03)	0.49 (0.05)	0.50 (0.10)
	% change[a]	–	16%	53%	2%	14%	−8%	−4%	−2%
	Female (n = 4–5)**								
	Mean (SD)	0.41 (0.04)	0.37 (0.04)	0.38 (0.06)	0.44 (0.01)	0.40 (0.04)	0.49 (0.08)	0.53 (0.04)	0.37 (0.05)
	% change[a]	–	−10%	−7%	7%	−2%	20%	29%	−10%
	Absolute thymus weight (g)								
	Male (n = 4–5)**								
	Mean (SD)	0.47 (0.08)	0.45 (0.08)	0.52 (0.17)	0.47 (0.07)	0.50 (0.09)	0.37 (0.06)	0.42 (0.09)	0.38 (0.13)
	% change[a]	–	−4%	11%	0%	6%	−21%	−11%	−19%
	Female (n = 4–5)								
	Mean (SD)	0.42 (0.06)	0.28 (0.10)	0.36 (0.09)	0.35 (0.07)	0.44 (0.07)	0.43 (0.08)	0.42 (0.08)	0.37 (0.10)
	% change[a]	–	−33%	−14%	−17%	5%	2%	0%	−12%
Hematology									
{Ema, 2008, 787657@@author-year} Rats, CRL:CD(SD) Diet Two generation F0: exposure started 10 wks prior to mating F1: maternal exposure throughout gestation/lactation; dietary exposure post weaning until necropsy	**Doses** (mg/kg-d)								
	Male, F0	**0**		**10**		**101**		**1,008**	
	Female, F0	**0**		**14**		**141**		**1,363**	
	Lymphocyte fraction (%)								
	Male, F0 (n = 10)								
	Response	88.5 (6.5)		88.8 (2.4)		88.8 (3.9)		87.5 (4.6)	
	% change[a]	–		0%		0%		−1%	
	Female, F0 (n = 10)								
	Mean (SD)	72.5 (8.7)		85* (5)		78.4 (9.5)		70.8 (9)	
	% change[a]	–		17%		8%		−2%	
	Segmented neutrophil fraction (%)								
	Male, F0 (n = 10)								
	Mean (SD)	8.00 (5.24)		8.24 (1.98)		7.68 (3.26)		8.68 (4.61)	
	% change[a]	–		3%		−4%		8%	
	Female, F0 (n = 10)								
	Mean (SD)	21.68 (8.08)		10.56* (4.19)		16.84 (9.19)		23.28 (8.13)	
	% change[a]	–		−51%		−22%		7%	
	Stab form neutrophil fraction (%)								
	Male, F0 (n = 10)								
	Mean (SD)	0.48 (0.73)		0.36 (0.3)		0.64 (0.28)		0.56 (0.51)	
	% change[a]	–		−25%		33%		17%	
	Female, F0 (n = 10)								

Reference and study design	Results							
	Mean (SD)	1.32 (0.57)	0.60* (0.39)		0.84 (0.55)		1.12 (0.7)	
	% change[a]	–	−55%		−36%		−15%	
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure starting on PNW 11	**Doses** (mg/kg-d)							
	Male	**0**	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	Lymphocyte cell fraction in blood (%)							
	Male (n = 3−5)							
	Mean (SD)	89.1 (2.5)	89.0 (3.7)	85.4 (5.9)	85.3 (2.0)	86.7 (3.7)	88.9 (3.8)	84.2 (8.1)	88.1 (3.1)
	% change[a]	–	0%	−4%	−4%	−3%	0%	−5%	−1%
Histopathology									
{van der Ven, 2006, 787745@@author-year} Rats, Wistar Gavage 28-d exposure starting on PNW 11	**Doses** (mg/kg-d)							
		0	**0.3**	**1**	**3**	**10**	**30**	**100**	**200**
	CD4 (Th) cells per spleen (cells $\times 10^7$)							
	Male (n =1−5)**							
	Mean (SD)	14.0 (4.7)	15.2 (n/a)	13.3 (4.8)	11.4 (n/a)	10.5 (0.9)	9.0 (n/a)	11.2 (n/a)	10.0 (2.0)
	% change[a]	–	9%	−5%	−19%	−25%	−36%	−20%	−29%
	Total immune cells per spleen (cells $\times 10^7$)								
	Male (n =1−5)**								
	Mean (SD)	48.7 (10.5)	49.6 (n/a)	47.1 (15.4)	44.4 (n/a)	39.4 (3.8)	29.7 (n/a)	37.0 (n/a)	35.8 (1.1)
	% change[a]	–	2%	−3%	−9%	−19%	−39%	−24%	−26%

*Statistically significantly different from the control at $p < 0.05$ as reported by study authors.
**Significant dose response trend as reported by study authors.
[a]Percent change compared to control calculated as: (treated value − control value)/control value × 100

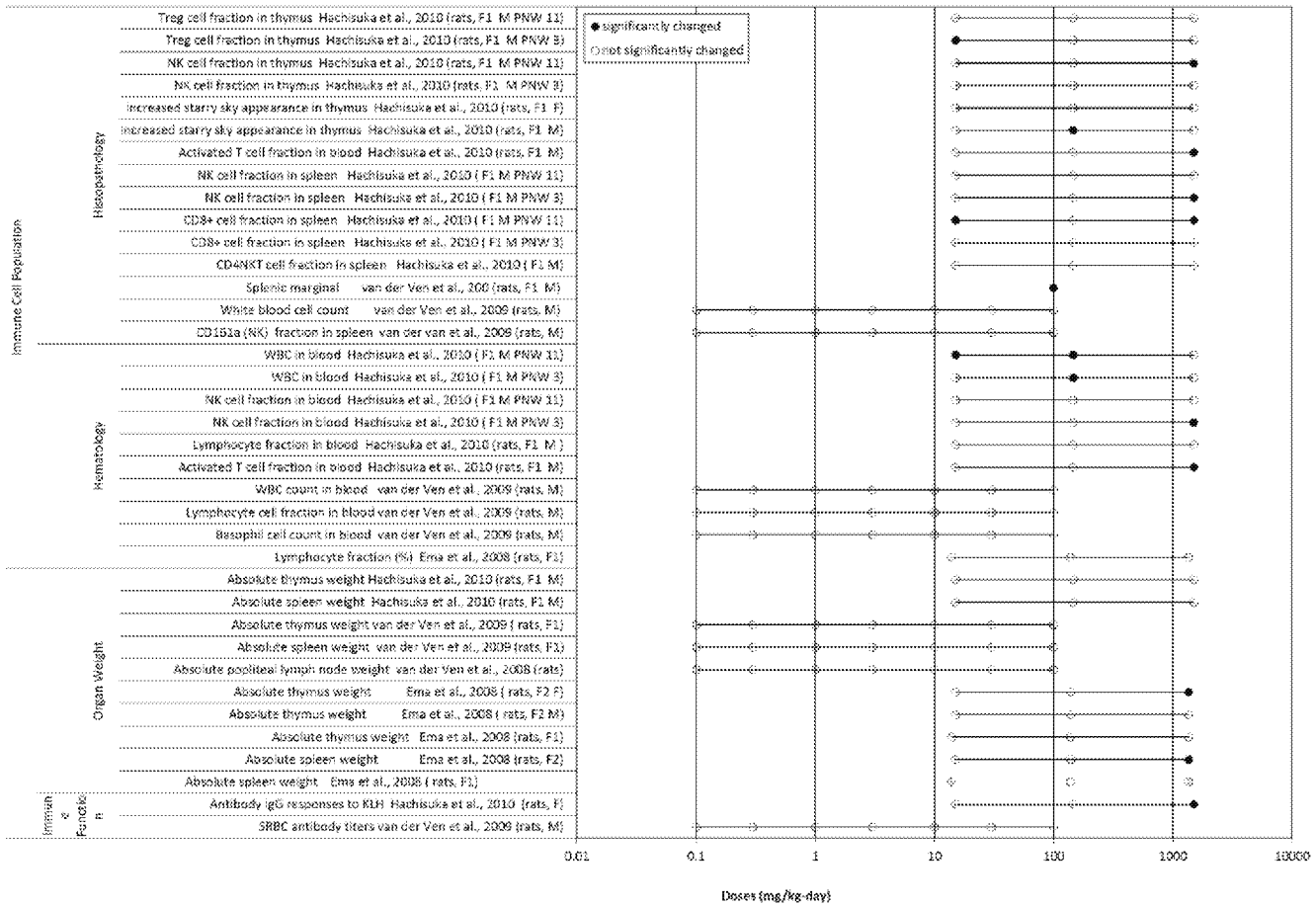


Figure 1-10. Exposure response array of immune system following oral exposure.

Commented [A2]: New ER arrays are housed in HAWC.

1.6.3. Mechanistic Evidence

Mechanistic information to support HBCD-mediated effects on the immune system is limited. Several recent in vitro studies in human immune cells suggest that HBCD may alter immune function through activation of MAPK signaling pathways (ERK1/2 and p38) resulting in increased secretion of IFN γ and IL-1β, pro-inflammatory cytokines that regulate immune function (Almighamsi, 2016; Anisuzzman, 2016; Cato, 2016). Similarly, pro-inflammatory effects driven by were observed in human brochial epithelial cells (BEAS-2B); HBCD exposure increased expression of proinflammatory cytokines (IL-6 and IL-8) and ICAM-1, a cell surface marker often expressed by immune cells, which were mediated by activation of MAPK signaling pathways (Koike, 2016). One study using human monocyte-derived dendritic cells found that co-exposure with HBCD enhanced IL-6 and IL-8 secretion elicited by environmental allergens (Canbaz, 2016).

{Koike, 2012, 1400827@@author-year} used bone marrow-derived dendritic cells prepared from atopic-prone NC/Nga mice to investigate HBCD effects on the immune response in vitro. HBCD (10 μg/mL) increased cell proliferation and expression of a dendritic activation marker, DEC205. Bone marrow-derived dentritic cells differentiated in the presence of HBCD also showed enhanced MHC class II, CD80, CD86, and CD11c expression. These in vitro data are supported by two studies using the guinea pig maximization test method that indicated that HBCD may act as a mild skin allergen {Momma, 1993, 1927836;Nakamura, 1994, 1928219}. Taken together, these studies suggest that HBCD may stimulate an immune response by increasing the activity of antigen-presenting cells. In vitro, HBCD altered several aspects of human NK cell function, including decreased target cell binding, expression of surface binding proteins, lytic function, and ATP levels {Hinkson, 2009, 1927711;Hinkson, 2010, 1927693}; however, in vivo NK cell activity was unaffected in rats {van der Ven, 2009, 589273;van der Ven, 2006, 787745}.

1.7. Genotoxicity

A limited number of studies have investigated the genotoxicity of HBCD; these are summarized in [REF _Ref532818975 \h * MERGEFORMAT]. The majority of these studies were standard Ames tests for detecting mutagenic potential in *Salmonella typhimurium*. These tests, which employ different strains of bacteria that have been developed with pre-existing mutations, including *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538, are referred to as reversion assays {Maron, 1983, 195187}. Most of these assays conducted with HBCD yielded negative results {IBT Labs, 1990, 787688; Litton Bionetics, 1990, 787698;SRI International, 1990, 787716;Zeiger, 1987, 699386;Huntingdon Research Centre, 1990,

787683;Pharmakologisches Inst, 1990, 787701}. Among the few assays performed to determine the genotoxicity of HBCD in prokaryotic systems, one in yeast {Litton Bionetics, 1990, 787698}, one detecting chromosomal aberrations in human peripheral lymphocytes in vitro {Microbiological Associates, 1996, 787699}, and one in vivo mouse micronucleus test following intraperitoneal (i.p.) injections of HBCD {BASF, 2000, 787637} were negative, even when tested at cytotoxic concentrations.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of genotoxicity studies of HBCD

Test/species/strain/ route	Test doses (per plate)[a]	Results[b]		Notes	Reference
		−S9	+S9		
Eukaryotic systems, in vitro					
S. typhimurium TA98, TA100, TA1535, TA1537	50–5,000 µg (HBCD bottoms) in acetone	+ (TA1535 and 100 only)	+ (TA100 only)	No cytotoxicity observed. Dose-response observed in TA1535 (−S9) ≥100 µg/plate. TA100 positive at highest dose only (5,000 µg/plate). All doses had a black precipitate thought to be carbon.	{Ethyl Corporation, 1990, 787661@@author-year}
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	50 µg (421–32B) (solvent not reported)	−	−		{Litton Bionetics, 1990, 787698@@author-year}
S. typhimurium TA98, TA100, TA1535, TA1537	2–1,000 µg (GLS-S6-41A) in DMSO	−	−		{GSRI, 1978, 1937197@@author-year}
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	100–10,000 µg in DMSO	−	−	Doses ≥1,000 µg were insoluble.	{Zeiger, 1987, 699386@@author-year}
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	250 µg (Firemaster, FM-100, Lot 53, white powder) in DMSO	−	−	Doses ≥250 µg were insoluble.	{IBT Labs, 1990, 787688@@author-year}
	1,000 µg (FM-100, Lot 3322, liquid residue)	−	+ (TA1535 only)	Significant in TA1535 at highest dose only.	

Test/species/strain/ route	Test doses (per plate)[a]	Results[b]		Notes	Reference
		−S9	+S9		
	in DMSO				
S. typhimurium TA98, TA100, TA1537	3,000 µg in DMSO	–	–	Doses ≥1,000 µg were partially insoluble.	{Pharmakologisc hes Inst, 1990, 787701@@author -year}
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	5,000 µg in DMSO	–	–	No cytotoxicity observed.	{SRI International, 1990, 787716@@author -year}
S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537	10,000 µg (Pyroguard SR-103) in DMSO	–	–		{Ogaswara, 1993, 2344713@@auth or-year}
S. typhimurium TA98, TA100, TA1535	10,000 µg in DMSO	–	–	Insoluble at 10,000 µg.	{Huntingdon Research Centre, 1990, 787683@@author -year}
Prokaryotic non-mammalian systems, in vitro					
Saccharomyces cerevisiae D4	50 µg (solvent not reported)	–	–		{Litton Bionetics, 1990, 787698@@author -year}
Mammalian systems, in vivo					
Micronucleus test mouse/NMRI/i.p. injection	2,000 mg/kg in DMSO	– (T)	NA	Toxicity evident as a slight inhibition of erythropoiesis at 2,000 mg/kg. Number of polychromatic erythrocytes with micronuclei from femoral bones evaluated 24 hrs after 2nd injection.	{BASF, 2000, 787637@@author -year}
Mammalian systems, in vitro					
Chromosomal aberration test Human peripheral blood lymphocytes	750 µg/mL (−S9) 250 µg/mL (+S9) in DMSO	– (T)	– (T)	Doses 750–2,500 µg/mL were partially insoluble, and fully insoluble >2,500 µg/mL.	{Microbiological Associates, 1996, 787699@@author -year}

Test/species/strain/ route	Test doses (per plate)[a]	Results[b]		Notes	Reference
		−S9	+S9		
				Repeated test for two harvest time points: 20-hr (−S9) or 4-hr (+S9) incubations, and 20- or 44-hr incubations (−S9 and +S9).	
Reversion assay CHO/V79/Sp5 and SPD8 Intragenic recombination at *hprt* locus in Sp5 (non-HR) and SPD8 (HR) duplication cell lines	3−20 µg/mL in DMSO	+	NA	A statistically significant, dose-dependent increase in reversion frequency was observed in both assays as determined by linear regression analysis. Significant inhibition of cloning efficiency occurred at doses ≥15 µg/mL in the SPD8 assay and ≥20 µg/mL in the Sp5 assay. Cytotoxicity (IC_{50}) measured at 0.02−0.03 mM.	{Helleday, 1999, 787680@@author-year}
Unscheduled DNA synthesis rat/F344 male/primary hepatocytes	10 µg/well in acetone (HBCD bottoms)	+	NA	Five highest doses (from 5 µg/well) showed an increased response with dose over solvent control, but only four highest were statistically significant (χ^2). Highest dose (1,000 µg/well) was cytotoxic.	{Ethyl Corporation, 1990, 1928253@@author-year}

[a]Lowest effective dose for positive results; highest dose tested for negative results.
[b]+ = positive; ± = equivocal or weakly positive; − = negative; T = cytotoxicity; NA = not applicable.

DMSO = dimethyl sulfoxide

Some positive results have been reported. *S. typhimurium* strain TA1535 was positive for reverse mutations at the highest dose only using a liquid residue of HBCD in DMSO {IBT Labs, 1990, 787688}, and strain TA100 was positive also at the highest dose using an unidentified mixture characterized only as HBCD bottoms in acetone {Ethyl Corporation, 1990, 787661}. In this same study, TA1535 was positive at ≥100 µg/plate without addition of an S9 microsomal

fraction {Ethyl Corporation, 1990, 787661}. The number of revertants increased with dose. This was the only Ames study to report dissolving the test article in a solvent other than DMSO (in this case, acetone). DMSO is a free-radical scavenger and can potentially obscure genetic damage due to oxidative radicals. Both strains TA1535 and TA100 were designed to be sensitive to detecting reversions by base substitution, a type of genetic lesion that can result from oxidative DNA damage due to reactive oxygen species (ROS). However, there is only limited evidence in the literature indicating that HBCD exposure may induce oxidative stress {An, 2013, 1927550;Hu, 2009, 837636}.

In mammalian systems, a reverse mutation assay with Chinese hamster ovary (CHO) Sp5 and SPD8 cell lines exposed to HBCD {Helleday, 1999, 787680} yielded positive results. These two clones exhibit a partial duplication of the hprt gene, causing lethality unless a reversion occurs, either via homologous recombination (SPD8) or non-homologous recombination (Sp5). A statistically significant, dose-dependent increase in reversion frequency was observed in both clones, although at higher doses, there was a significant inhibition of cloning efficiency. In addition, a test of unscheduled DNA synthesis with rat hepatocytes exposed to HBCD bottoms was positive {Ethyl Corporation, 1990, 1928253}, and also showed an increase in response with dose.

It is noteworthy that in these three studies {Helleday, 1999, 787680}, the positive results were dose-dependent, observed at nontoxic doses, and in two assays, specific for detecting mutations. However, the Ames tests in the same strains that showed positive results (TA1535 and TA100) were negative in seven other studies, and the results in the reverse mutation assay in CHO cells {Helleday, 1999, 787680} have not been confirmed by another group.

2. DOSE-RESPONSE ANALYSIS

2.1. Supplemental Information on Non-Cancer Dose Response Analysis

2.1.1. Additional Considerations for Selection of Studies for Dose-Response Analysis

As discussed in Section 1.3.2, HBCD is likely to cause thyroid toxicity in humans, and there is suggestive evidence of liver, female reproductive, and developmental toxicity following oral exposure to hexabromocyclododecane (HBCD). These hazards have been carried forward for dose-response analysis. There is also suggestive evidence of nervous system toxicity following exposure to HBCD; however, for the reasons discussed in Section 1.3.2, data sets representative of this hazard were not carried forward for dose-response analysis.

The effects determined to best represent each of the hazards were identified in Section 1.3.2, and studies that evaluated these effects are considered in this section for dose-response analysis. In order to identify the stronger studies for dose-response analysis, several attributes of the studies were reviewed. Preference was given to studies using designs reasonably expected to detect a dose-related response. Chronic or subchronic studies are generally preferred over studies of less-than-subchronic duration for deriving chronic and subchronic reference values. Studies with a broad exposure range and multiple exposure levels are preferred to the extent that they can provide information about the shape of the exposure-response relationship. Additionally, with respect to measurement of the endpoint, studies that can reliably measure the magnitude and/or degree of severity of the effect are preferred.

Human studies are generally preferred over animal studies as the basis for a reference value when quantitative measures of exposure are available and the reported effects are determined to be associated with exposure because they remove uncertainties associated with extrapolation across species. As discussed in earlier sections, studies in humans were not adequate to support conclusions regarding the relationship between HBCD exposure and effects on the thyroid, male reproduction, or nervous system, and accordingly do not support dose-response analysis. In the absence of human data, animal toxicity studies were used for dose-response analysis.

Experimental animal studies considered for each hazard and effect were evaluated using general study quality considerations discussed above and in the Systematic Review Methods section. The rationales for selecting the strongest studies to represent these hazards are summarized below.

1

2

2.1

2.1.1

2.1.1.1 Thyroid Effects

Regulation of thyroid hormones is complex and homeostasis is largely maintained via HPT axis feedback mechanisms. Reductions in serum T3 or T4 triggers release of TSH from the pituitary, which stimulates the thyroid gland to increase secretion of T3 and T4 stores from the colloid {Fisher, 2012, 3042123}. Decreased T4 is expected to be the primary driver of HBCD-mediated thyroid effects that triggers release of TSH. Indeed, this is supported by mechanistic studies that indicate that that observed decreases in T4 may be largely driven by hepatic induction of enzymes that metabolize this hormone (See Section [REF _Ref2929776 \r \h], Mechanistic Evidence).

Overall, HBCD is likely to cause thyroid toxicity in humans. Despite demonstrating a sensitive response to HBCD exposure, follicle size was not selected for modeling because: (1) quantitative data for follicle size changes were provided only in one study (Ema, 2008); (2) although this is generally a well conducted

study, details of the methods of analysis (e.g., the criteria used to determine whether an animal showed decreased follicle size) were not provided; and (3) although changes in thyroid histopathology (e.g., follicle size, epithelial cell hypertrophy) can be useful indicators of changes in thyroid function/homeostasis, they are less direct measures of thyroid toxicity and it would be difficult to determine an appropriate benchmark response (BMR).

Serum thyroxine (T4) was selected for dose-response analysis of thyroid effects (see Section 1.3.2). Three studies in rats reported treatment-related decreases in serum T4 following oral exposure {Ema, 2008, 787657;van der Ven, 2006, 787745;WIL Research, 2001, 787787}. Table 2-1 provides an overview of the study designs for those studies reporting T4 levels that were evaluated for dose-response analysis.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Study design features of studies that examined T4 levels

Study reference	Route	Exposure duration	Number of dose groups[a]	Number of animals/group	Dose range (mg/kg-d)
{Ema, 2008, 787657@@author-year}	Diet	Two-generation	3	8 rats/sex	10–1,363[a]
{WIL Research, 2001, 787787@@author-year}	Gavage	90 d	3	5–10 rats/sex	100–1,000
{van der Ven, 2006, 787745@@author-year}	Gavage	28 d	7	4–5 rats/sex	0.3–200

[a]Doses differed by sex and generation (see, for example, Table 1-3).

{Ema, 2008, 787657@@author-year} reported a decrease in serum T4 levels in both male and female rats from the F0 (30 and 31% at the high dose, respectively) and F1 (10 and 28% at the high dose, respectively) generations. {van der Ven, 2006, 787745@@author-year} reported similar effects on serum T4 (26% reduction at the high dose) in adult female rats exposed for 28 days. {WIL Research, 2001, 787787@@author-year} reported changes in T4 levels in rats exposed to HBCD for 90 days, but inadequate reporting of thyroid hormone measurement methods, high proportion (50%) of samples below the limit of detection, and unusually low control thyroid-stimulating hormone (TSH) levels reduced the confidence in these results, bringing into question the conduct of the assays.

Based on considerations of study design and magnitude of T4 response, T4 data sets from {Ema, 2008, 787657@@author-year} were selected for dose-response analysis. The 2-generation study design used by {Ema, 2008, 787657@@author-year} involved a longer exposure duration and larger group size than {van der Ven, 2006, 787745@@author-year}. Specifically, T4 data from F0 male and female rats and F1 female rats from {Ema, 2008, 787657@@author-year} were used for quantitative analysis. Because the magnitude of response in F1 male rats was smaller than F0 male and female rats and F1 females (by one-third to one-half), T4 data from this group of animals was not modeled.

2.1.1.2 Liver Effects

The most consistently observed liver outcome was liver weight changes. Dose-related increases were consistently observed across species, sexes, and age from multiple studies of various designs and exposure durations {Yanagisawa, 2014, 2343717; Maranghi, 2013, 1927558;Saegusa, 2009, 787721;Ema, 2008, 787657;WIL Research, 1997, 787758;WIL Research, 2001, 787787}. Limited support for HBCD effects on the liver are provided by histopathological examination. A subset of the rat studies {Saegusa, 2009, 787721; WIL Research, 1997, 787758;WIL Research, 2001, 787787} and one mouse study {Maranghi, 2013, 1927558} reported increased vacuolation (generally of minimal to mild severity) in HBCD-exposed animals, but these responses were not dose-related. The content of the vacuoles was investigated only by {WIL Research, 2001, 787787@@author-year} and characterized as lipid. Other histological findings were less frequently observed and included some additional evidence of fatty change (steatosis) {Yanagisawa, 2014, 2343717}, hypertrophy {Yanagisawa, 2014, 2343717;WIL Research, 1997, 787758}, and inflammation {Maranghi, 2013, 1927558}. Statistically or biologically significant elevations in serum liver enzymes were not associated with HBCD exposure in rats or mice in multiple studies {WIL Research, 2001, 787787;WIL Research, 1997, 787758;Yanagisawa, 2014, 2343717}, however in contrast mechanistic evidence in vitro suggests that HBCD may in fact induce hepatic microsomal enzymes {Germer, 2006, 787665;Crump, 2008, 1408111;Crump, 2010, 1403482}. Microsomal enzyme induction is a proposed key event in initiating the perturbation of the HPT axis that leads to reduced T4 levels. Given limited evidence of HBCD-related histopathological changes and no clear evidence of clinical chemistry changes, the biological significance of liver weight changes is unclear. While increased liver weight was not consistently associated with other toxicological evidence of liver toxicity in rodents given a standard diet, biochemical and histopathological effects indicative of steatosis were observed in mice fed a high-fat diet {Yanagisawa, 2014, 2343717}. A high-fat diet may therefore represent a susceptibility factor for TCE toxicity {Bernhard, 2016, 3545918}.

Increased liver weight was selected for dose-response analysis of liver effects (see Section 1.3.2). This endpoint was reported in six studies in rats {Ema, 2008, 787657;WIL Research, 2001, 787787;van der Ven, 2006, 787745;Saegusa, 2009, 787721;WIL Research, 1997, 787758} and mice {Maranghi, 2013, 1927558}. Table 2-2 provides an overview of the study designs for those studies reporting relative liver weight that were evaluated for dose-response analysis.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Study design features of studies that examined liver weight

Study reference	Route	Exposure duration	Number of dose groups[a]	Number of animals/group	Dose range (mg/kg-d)
{Ema, 2008, 787657@@author-year}	Diet	Two-generation	3	13–24 rats/sex	10–1,570[a]
{WIL Research, 2001, 787787@@author-year}	Gavage	90 d	3	10 rats/sex	100–1,000
{van der Ven, 2006, 787745@@author-year}	Gavage	28 d	7	4–5 rats/sex	0.3–200
{WIL Research, 1997, 787758@@author-year}	Gavage	28 d	3	6 rats/sex	125–1,000
{Saegusa, 2009, 787721@@author-year}	Diet	Gestation and lactation (~42 d)	3	10 rats/sex	15–1,505

Study reference	Route	Exposure duration	Number of dose groups[a]	Number of animals/group	Dose range (mg/kg-d)
{Maranghi, 2013, 1927558@@author-year}	Diet	28 d	1	10–15 female mice	199

[a]Doses differed by sex and generation (see, for example, Table 1-4).

The developmental study by {Saegusa, 2009, 787721@@author-year} and the 28-day study by {WIL Research, 1997, 787758@@author-year} used similar dose ranges as the longer-duration studies {Ema, 2008, 787657;WIL Research, 2001, 787787} and observed similar findings in pup or adult liver weights. A significant trend in increased liver weight was reported by {van der Ven, 2006, 787745@@author-year} following a 28-day adult exposure at lower doses, but in female rats only. Data from these shorter exposure duration studies were not used for dose-response analysis because similar effects were observed in the studies with longer exposure durations {Ema, 2008, 787657;WIL Research, 2001, 787787} that better reflect effects expected following subchronic or chronic exposure. Similarly, {Maranghi, 2013, 1927558@@author-year} was not used for dose-response analysis because it used a relatively short (28-day) exposure and a single dose group that is less informative for evaluating a dose-response relationship.

2.1.1.3 Female Reproductive Effects

See the Risk Evaluation document for details on this endpoint.

2.1.1.4 Developmental Effects

Several studies in animals exposed during gestation and lactation provide some evidence of developmental effects associated with HBCD, including reduced offspring viability {Ema, 2008, 787657}, decreased pup body weight {van der Ven, 2009, 589273; Ema, 2008, 787657;Saegusa, 2009, 787721;Maranghi, 2013, 1927558}, altered development of the skeletal system, and delayed eye opening {Ema, 2008, 787657}. The strongest evidence of developmental effects is based on findings of reduced offspring viability and decreased pup body weight. Reduced viability was observed in the two-generation study by {Ema, 2008, 787657@@author-year}; the decreases in viability were dose-related and observed on both PND 4 and 21. That effects were seen only in F2 offspring is consistent with decreased viability manifesting after multigenerational exposure, although that hypothesis cannot be established based on the current developmental literature for HBCD (i.e., a single two-generation study). Effects on pup body weight were demonstrated in several studies in rats using different strains and exposure durations {van der Ven, 2009, 589273; Ema, 2008, 787657; Saegusa, 2009, 787721}. Other developmental effects, including changes in bone development and delayed eye opening, were only reported in a single study and with a less clear dose-response relationship {van der Ven, 2009, 589273;Ema, 2008, 787657}. Therefore, pup body weight and viability were selected for dose-response analysis of developmental effects. Table 2-3 summarizes study design features considered in evaluating the strength of each study that reported changes in pup weight for purpose of dose-response analysis.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Study design features of studies that examined pup weight

Study reference	Route	Exposure duration	Number of dose groups[a]	Number of animals/group	Dose range (mg/kg-d)
{Ema, 2008, 787657@@author-year}	Diet	Two-generation	3	13–24 rat litters	10–1,570[b]
{van der Ven, 2009, 589273@@author-year}	Diet	One-generation	7	≥14 rats	0.1–100
{Saegusa, 2009, 787721@@author-year}	Diet	Gestation and lactation (~42 d)	3	10–14 rats/sex[c]	15–1,505
{Maranghi, 2013, 1927558@@author-year}	Diet	28 d	1	10–15 female mice	199

[a]Excludes the control group.
[b]Doses differed by sex and generation (see, for example, Table 1-4).
[c]For PND 0 data, exact number of animals examined per dose group was unclear based on the published study.

{Ema, 2008, 787657@@author-year} evaluated changes in pup body weight in rats that were continuously exposed to HBCD across two generations. Treatment-related effects on pup body weight were measured throughout early postnatal development (PNDs 0, 4, 7, 14, and 21) in three dose groups, covering a dose range of approximately 2.5 orders of magnitude. This study used an adequate sample size (n = 13–24) and litter as the statistical unit. {Maranghi, 2013, 1927558@@author-year} was considered less appropriate to support derivation of an RfD because the study used only one dose group, which is less informative for evaluating dose-response relationships, and a relatively short exposure duration (28 days). {van der Ven, 2009, 589273@@author-year} used a dose range that was >10-fold lower than those used in the {Ema, 2008, 787657@@author-year} and {Saegusa, 2009, 787721@@author-year} studies and, in general, did not show a clear pattern of dose-related changes in pup body weight on different days of lactation.

2.1.2. BMR Selection

A set of dose-response models that are consistent with a variety of potentially underlying biological processes were applied to empirically model the dose-response relationship in the range of the observed data. The models in EPA's Benchmark Dose Software (BMDS, version 2.6) were applied. Consistent with EPA's *Benchmark Dose Technical Guidance Document* {U.S. EPA, 2012, 1239433}, the benchmark dose (BMD) and 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) to represent a minimal, biologically significant level of change, described here as relative deviation (RD). In the absence of information regarding the level of change that is considered biologically significant, a BMR of 1 standard deviation (SD) from the control mean for continuous data or a BMR of 10% extra risk (ER) for dichotomous data is used to estimate the BMD and BMDL, and to facilitate a consistent basis of comparison across endpoints, studies, and assessments. Endpoint-specific BMRs are described further below. Where modeling was feasible, the estimated BMDLs were used as points of departure (PODs). Further details, including the modeling output and

graphical results for the model selected for each endpoint, can be found in Appendix D. Where dose-response modeling was not feasible, NOAELs or LOAELs were identified and are summarized in Table 2-4.

2.1.1.5 Thyroid Effects

Changes in T4 levels described by {Ema, 2008, 787657@@author-year} were amenable to BMD modeling. In selecting a BMR (i.e., a change in T4 levels considered biologically significant), pregnant females and their offspring were addressed separately from adult males. Early life development is generally recognized as being particularly sensitive to thyroid perturbation. Thyroid hormones play a critical role in coordinating complex developmental processes, and perturbations of thyroid hormone levels in a pregnant woman or neonate can have persistent adverse health effects for the child. During early gestation, the developing fetus relies solely on thyroid hormones of maternal origin. As the fetus begins to produce thyroid hormones, there is less reliance on maternal thyroid hormones; however, early development remains a sensitive life stage for hormone deficits, largely due to minimal reserve capacity when compared to adults {Gilbert, 2010, 3449218}.

Reductions in maternal T4 during pregnancy or the early postnatal period are strongly associated with adverse neurological outcomes in offspring. In humans, mild to moderate maternal thyroid insufficiency is associated with higher risk for persistent cognitive and behavioral deficits in children. In general, mild to moderate thyroid insufficiency in pregnant women was defined as serum T4 levels below the 10th percentile for the study population, which is associated with a 15–30% decrease relative to the corresponding median {Henrichs, 2010, 758743;Haddow, 1999, 2176;Finken, 2013, 3116021;Román, 2013, 3121313;Julvez, 2013, 3421483}. Similar effects have been described in animal studies, with modest reductions in maternal T4 during gestation resulting in behavioral alterations, learning deficits, and neuroanatomical changes in offspring {Gilbert, 2011, 1247910;Gilbert, 2013, 2163506;Gilbert, 2014, 3043020;Ausó, 2004, 627573;Liu, 2010, 755845}. Thyroid inhibition during gestation and lactation that resulted in drops in mean maternal T4 levels of ~10–17% have been found to elicit neurodevelopmental toxicity in offspring {Gilbert, 2011, 1247910;Gilbert, 2016, 3421484}. Although there are some differences in HPT regulation (e.g., serum hormone binding proteins, hormone turnover rates, and timing of in utero thyroid development), rodents are generally considered to be a good model for evaluating the potential for thyroid effects of chemicals in humans {Zoeller, 2007, 3456414}. Based on the data observed in both humans and animals, a BMR of 10% RD from control mean was determined to be a minimally biologically significant degree of change when performing BMD modeling using female rat data.

The available thyroid literature does not support identification of a biologically significant change in T4 levels in adult males as decreases in T4, and more generally thyroid function, have not been conclusively linked to similarly severe outcomes as in females. Nevertheless, males with depressed T4 values are part of the subpopulation that experiences thyroid dysfunction. Selecting a biologically-based BMR is also complicated by the inherent variability of thyroid hormones. Individuals show relatively narrow variability around a set point; however, set points can vary considerably between individuals, resulting in a broad population range that is considered normal {Andersen, 2002, 51721}. Thus, it is

possible for an individual to have thyroid levels that fall within the normal population range, but are abnormal relative to their homeostatic set point. Consistent with EPA's *Benchmark Dose Technical Guidance Document* {U.S. EPA, 2012, 1239433}, a BMR of one control SD change from the control mean was applied in modeling T4 data from male rats in the absence of a biological basis for selecting a BMR.

Additionally, a BMR of 10% RD from control means, supported by the literature on the effects of thyroid insufficiency in pregnant females and their offspring, was applied in modeling the male T4 data. In looking across the available HBCD studies, there does not appear to be a strong sex-specific effect on T4 responses (see Table 1-3). Differences in dose-response (i.e., similar responses at the high dose but divergent responses at the lower doses) was observed in the F0 male and female data sets that were modeled {Ema, 2008, 787657}. These differences likely reflect the inherent variability of thyroid hormones within a population, especially for a relatively small sample size as used in {Ema, 2008, 787657@@author-year}, and not a sex-specific difference in response. Under the assumption that differences in thyroid hormone response in male and female rats exposed to HBCD are not sex-specific but rather a reflection of hormone variability, using a BMR of 10% RD was considered reasonable.

2.1.1.6 Liver Effects

See the Risk Evaluation document for details on this endpoint.

Commented [A3]: NOTE: In response to a question raised by Keith Jacobs, we plan to revise this section to better support our rationale for the studies we used for dose-response analysis.

2.1.1.7 Female Reproductive Effects

2.1.1.7.1 Primordial follicle count

Decreased primordial follicle count as reported in the two-generation reproductive toxicity study by {Ema, 2008, 787657@@author-year} was amenable to BMD modeling. Because primordial follicles are formed during gestation, the average dose during this critical window was used for BMD modeling. A BMR of 10% RD from control levels was applied in modeling this endpoint under the assumption that it represents a minimal biologically significant effect. There is no consensus in the scientific community regarding the degree of change that is considered to be adverse. In this situation, it has been suggested that a detectable decrease in follicle number should be considered adverse {Heindel, 1998, 3393147}. Power analyses by {Heindel, 1998, 3393147@@author-year} focused on identifying follicle counts reduced by ≥20%, suggesting that a reduction of this magnitude is considered a critical effect level. Thus, a 10% reduction was selected to represent a minimally important degree of change.

2.1.1.7.2 Pregnancy incidence

In the study by {Ema, 2008, 787657@@author-year}, the increased incidence of non-pregnancy in HBCD-exposed F0 or F1 rats alone was not statistically significant with either pairwise test (as reported by authors) or Cochran-Armitage trend test (conducted by EPA). Dose-response curves were shallow and never reached a high response percentage. To increase statistical power and obtain a more precise estimate of the BMD and BMDL, consideration was given to combining F0 and F1 datasets. Cochran-Mantel-Haenszel statistics on F0 and F1 data stratified by dose groups were not significant ($p = 0.59$, $\alpha = 0.05$), indicating no statistical association between generation and response after adjusting for

dose. Equality of responses in F0 and F1 rats was also not rejected ($p > 0.2$, $\alpha = 0.05$) by the Breslow-Day test for homogeneity of the odds ratios, and their background response percentages were not detectably different (Fisher's exact, $p = 1.00$). The results of these statistical tests suggested that F0 and F1 datasets were compatible for combining. A statistically significant trend ($p = 0.02$) was found using the Cochran-Armitage test applied to the combined data. The Log-logistic model was selected after dropping the highest dose (see Supplemental Information, Appendix D, Section D.2). F0 and F1 data were also modeled separately after dropping the highest dose. A Likelihood ratio test ($\alpha = 0.05$, d.f. = 3) could not reject equality of the three Log-logistic models from combined dataset and F0, F1 alone. Therefore, the Log-logistic model from the combined dataset was used to derive the BMD and BMDL for increased incidence of non-pregnancy with increasing dose.

A BMR of 5% ER was applied in modeling this endpoint under the assumption that it represents a minimal biologically significant degree of change. Selection of a BMR took into consideration the limited sensitivity of rodent species to effects on fertility and pregnancy outcomes {U.S. EPA, 1996, 30019}. As noted in {U.S. EPA, 1996, 30019@@author-year}, the limited sensitivity of fertility measures in rodents suggests that a POD (i.e., NOAEL, LOAEL, or BMD) based on fertility may not reflect completely the extent of effects on reproduction, such that the BMD may need to be adjusted to reflect that additional uncertainty. Rather than applying an additional uncertainty factor to the POD based on reduced fertility in rats, a BMR of 5%, rather than 10%, was selected. A BMR of 5% ER was also consistent with the functional severity of the endpoint (i.e., reduced fertility).

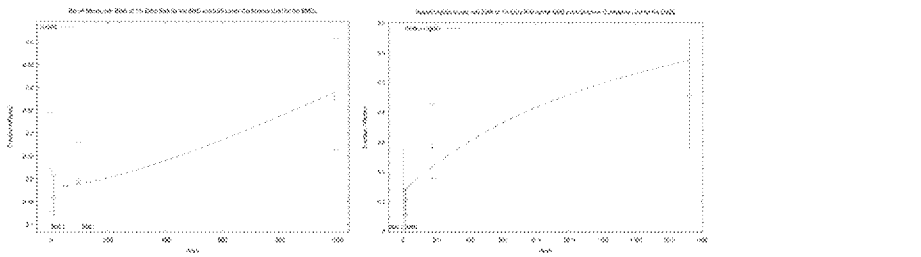
2.1.1.8 Developmental Effects

2.1.1.8.1 Offspring loss

Increased offspring loss in the F2 generation from the {Ema, 2008, 787657@@author-year} study was amenable to BMD nested modeling, using individual animal data obtained from the study authors (personal communication) {Makris, 2016 #271}. Two datasets were modeled: offspring loss from implantation through PND 4 and offspring loss from PND 4 (post-culling) through PND 21. Maternal gestational doses (10, 100, and 995 mg/kg-day) were used to model the offspring loss from the implantation through PND 4 dataset because they are reflective of the majority of the exposure window being modeled (i.e., 3 weeks of gestation compared to 4 days of lactation) and early lactational doses are closer to the gestational doses than the average dose during the entire lactational period. For similar reasons, modeling for the PND 4 post-culling through PND 21 dataset was performed using the maternal lactational doses (20, 179, and 1,724 mg/kg-day). Use of maternal lactational doses for modeling the PND 4 to 21 dataset was also consistent with total litter loss in eight high-dose dams that occurred at time points across the lactational period (specifically, PNDs 4, 5, 7, 9, 11, 13, and 18).

From a statistical standpoint, most reproductive and developmental studies with nested study designs can easily support a BMR of 5% ER {U.S. EPA, 2012, 1239433}. A smaller BMR of 1% ER was used in this case to address the severity of this endpoint (i.e., offspring loss). The use of a 1% ER BMR for offspring loss as reported in {Ema, 2008, 787657@@author-year} resulted in $BMDL_{01}$ values for loss from implantation through PND 4 and for offspring loss from PND 4 post-culling through

PND 21 in F2 rats that fell in the region of the dose-response curve where the response in dosed animals was similar to the response in the controls (see Figure 2-1).



A B

Figure 2-1. BMD modeling plots of incidence of offspring loss from implantation through PND 4 in F2 offspring rats (A) and incidence of offspring loss from PND 4 post-culling through PND 21 in F2 offspring rats (B) from {Ema, 2008, 787657@@author-year}; BMR = 1% ER (see Appendix D, Figures D-31 and D-33).

A NOAEL was also considered as the POD in addition to the POD derived using a BMD modeling approach. As shown in Figure 2-1, there is variation around the response at each dose. Although the responses at the $BMDL_{01}$ for each data set modeled appear not to be elevated over the control, the possibility of a small increase in response at these dose levels cannot be eliminated. Because the BMD approach is generally preferred to the NOAEL/LOAEL approach, and because the $BMDL_{01}$ values are similar to the NOAELs (difference of approximately 2-fold), the $BMDL_{01}$ values were used to estimate the PODs for offspring loss. For purposes of comparison, a POD based on the NOAEL is presented in addition to the $BMDL_{01}$ (see Table 2-4).

2.1.1.8.2 Pup body weight

Changes in F2 pup body weight as reported in the two-generation reproductive toxicity study by {Ema, 2008, 787657@@author-year} were amenable to BMD modeling. The average maternal exposures during lactation only (F2 = 20, 179, and 1,724 mg/kg-day) were used for modeling because no treatment-related effects were observed at birth, suggesting that decreases in pup body weight were driven by lactational exposure. A BMR of 5% RD from control mean was applied in modeling pup body weight changes under the assumption that it represents a minimal biologically significant response. Decreased body weight or body weight gain during the perinatal period is considered to be indicative of altered growth, an effect that has been identified as one of the four manifestations of developmental toxicity {U.S. EPA, 1991, 8567}. In adults, a 10% decrease in body weight in animals is generally recognized as a biologically significant response associated with identifying a maximum tolerated dose; during development, however, identification of a smaller (5%) decrease in body weight is consistent with the assumptions that development represents a susceptible lifestage and that the developing animal is more

adversely affected by a decrease in body weight than the adult. In humans, reduced birth weight is associated with numerous adverse health outcomes, including increased risk of infant mortality as well as heart disease and type II diabetes in adults {Barker, 2007, 451407;Reyes, 2005, 1065677}. For these reasons, a BMR of 5% RD was selected for decreased pup weight.

3. DOSE-RESPONSE MODELING FOR THE DERIVATION OF REFERENCE VALUES FOR EFFECTS OTHER THAN CANCER AND THE DERIVATION OF CANCER RISK ESTIMATES

This appendix provides technical detail on dose-response evaluation and determination of points of departure (PODs) for relevant toxicological endpoints. The endpoints were modeled using the U.S. Environmental Protection Agency (EPA) Benchmark Dose Software (BMDS, version 2.6). This appendix describes the common practices used in evaluating the model fit and selecting the appropriate model for determining the POD, as outlined in the Benchmark Dose Technical Guidance Document {U.S. EPA, 2012, 1239433}. In some cases, it may be appropriate to use alternative methods, based on statistical judgment; exceptions are noted as necessary in the summary of the modeling results.

3.1. NONCANCER ENDPOINTS

The noncancer endpoints that were selected for dose-response modeling are presented in [REF _Ref532886347 \h]For each endpoint, the doses and response data used for the modeling are presented.

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Noncancer endpoints selected for dose-response modeling for HBCD

Endpoint	Species (strain)/sex	Dose (mg/kg-d)[a]	Incidence [%] or mean ± SD (number of animals or litters)	BMR(s)
Thyroid				
↓T4 {Ema, 2008, 787657@@author-year}	F0 rats (CRL Sprague-Dawley)/male	0 10 101 1,008 TWA of lifetime exposure, F0	4.04 ± 1.42 (8) 3.98 ± 0.89 (8) 2.97 ± 0.76 (8) 2.49 ± 0.55 (8)	10% RD, 15% RD, 20% RD, 1 SD
↓T4 {Ema, 2008, 787657@@author-year}	F0 rats (CRL Sprague-Dawley)/female	0 14 141 1,363 TWA of lifetime exposure, F0	2.84 ± 0.61 (8) 3.14 ± 0.48 (8) 3.00 ± 0.77 (8) 1.96 ± 0.55 (8)	10% RD, 15% RD, 20% RD, 1 SD
↓T4 {Ema, 2008, 787657@@author-year}	F1 rats (CRL Sprague-Dawley)/female	0 14.3 138 1,363	3.59 ± 1.08 (8) 3.56 ± 0.53 (8) 3.39 ± 1.21 (8) 2.58 ± 0.37 (8)	10% RD, 15% RD, 20% RD, 1 SD

Endpoint	Species (strain)/sex	Dose (mg/kg-d)[a]	Incidence [%] or mean ± SD (number of animals or litters)	BMR(s)
		TWA of lifetime exposure, F1		
Liver				
Relative liver weight {Ema, 2008, 787657@@author-year}	F1 rats (CRL Sprague-Dawley)/male weanlings, PND 26	0 16.5 168 1,570 TWA of F0 gestational and lactational doses	4.6 ± 0.37 (23) 4.6 ± 0.32 (21) 5.05 ± 0.32 (20) 6 ± 0.44 (17)	10% RD, 1 SD
Relative liver weight {Ema, 2008, 787657@@author-year}	F1 rats (CRL Sprague-Dawley)/female weanlings, PND 26	0 16.5 168 1,570 TWA of F0 gestational and lactational doses	4.57 ± 0.35 (23) 4.59 ± 0.28 (21) 5.02 ± 0.32 (20) 6.07 ± 0.36 (14)	10% RD, 1 SD
Relative liver weight {Ema, 2008, 787657@@author-year}	F1 rats (CRL Sprague-Dawley)/male adults	0 11.4 115 1,142 TWA of lifetime exposure, F1	3.27 ± 0.18 (24) 3.34 ± 0.26 (24) 3.37 ± 0.25 (22) 3.86 ± 0.28 (24)	10% RD, 1 SD
Relative liver weight {Ema, 2008, 787657@@author-year}	F1 rats (CRL Sprague-Dawley)/female adults	0 14.3 138 1,363 TWA of lifetime exposure, F1	4.18 ± 0.42 (22) 4.39 ± 0.44 (22) 4.38 ± 0.47 (20) 5.05 ± 0.50 (13)	10% RD, 1 SD
Relative liver weight {Ema, 2008, 787657@@author-year}	F2 rats (CRL Sprague-Dawley)/male weanlings, PND 26	0 14.7 139 1,360 TWA of F1 gestational and lactational doses	4.72 ± 0.59 (22) 4.74 ± 0.35 (22) 5.04 ± 0.4 (18) 6.0 ± 0.25 (13)	10% RD, 1 SD
Relative liver weight {Ema, 2008, 787657@@author-year}	F2 rats (CRL Sprague-Dawley)/female weanlings, PND 26	0 14.7 139 1,360 TWA of F1 gestational and lactational doses	4.70 ± 0.27 (21) 4.70 ± 0.28 (22) 4.94 ± 0.32 (20) 5.89 ± 0.44 (13)	10% RD, 1 SD
Relative liver weight	Rats (Sprague-Dawley)/male	0 100	2.709 ± 0.1193 (10) 3.175 ± 0.2293 (10)	10% RD, 1 SD

Endpoint	Species (strain)/sex	Dose (mg/kg-d)[a]	Incidence [%] or mean ± SD (number of animals or litters)	BMR(s)
{WIL Research, 2001, 787787@@author-year}		300 1,000	3.183 ± 0.2653 (10) 3.855 ± 0.1557 (9)	
Relative liver weight {WIL Research, 2001, 787787@@author-year}	Rats (Sprague-Dawley)/female	0 100 300 1,000	2.887 ± 0.2062 (10) 3.583 ± 0.2734 (10) 3.578 ± 0.3454 (10) 4.314 ± 0.2869 (10)	10% RD, 1 SD
Reproductive				
Primordial follicles {Ema, 2008, 787657@@author-year} (supplemental)	F1 parental rat (CRL Sprague-Dawley)/female	0 9.6 96 941 The F0 adult female gestational doses	316.3 ± 119.5 (10) 294.2 ± 66.3 (10) 197.9 ± 76.9 (10) 203.4 ± 79.5 (10)	1% ER, 5% ER, 10% ER
Incidence of non-pregnancy {Ema, 2008, 787657@@author-year}	F0 and F1 parental rats combined (CRL Sprague-Dawley)/female	0 13.3 132 1,302 TWA F0, F1 female pre-mating doses	1/48 [2%] 3/48 [6.2%] 7/48 [14.5%] 7/47 [14.9%]	5% ER, 10% ER
Developmental				
Offspring loss at PND 4 {Ema, 2008, 787657@@author-year}	F2 offspring rats (CRL Sprague-Dawley)	0 9.7 100 995 The F1 adult female gestational doses	28/132 [21%] 26/135 [19.3%] 23/118 [19.5%] 47/120 [39.2%]	1% ER, 5% ER
Offspring loss at PND 21 {Ema, 2008, 787657@@author-year}	F2 offspring rats (CRL Sprague-Dawley)	0 19.6 179 1,724 The F1 adult female lactational doses	11/70 [15.7%] 7/70 [10.0%] 18/64 [28.1%] 32/64 [50.0%]	1% ER, 5% ER
Pup weight during lactation at PND 21 {Ema, 2008, 787657@@author-year}	F2 offspring rats (CRL Sprague-Dawley)/male	0 19.6 179 1,724 The F1 adult female lactational doses	53 ± 12.6 (22) 56.2 ± 6.7 (22) 54.1 ± 10.1 (18) 42.6 ± 8.3 (13)	5% RD, 10% RD, 0.5 SD, 1 SD
Pup weight during lactation at PND 21	F2 offspring rats (CRL Sprague-Dawley)/female	0 19.6 179	52 ± 10 (21) 52.8 ± 6.6 (22) 51.2 ± 10.8 (20)	5% RD, 10% RD,

Endpoint	Species (strain)/sex	Dose (mg/kg-d)[a]	Incidence [%] or mean ± SD (number of animals or litters)	BMR(s)
{Ema, 2008, 787657@@author-year}		1,724 The F1 adult female lactational doses	41.6 ± 8.4 (13)	0.5 SD, 1 SD

[a]Doses were calculated as TWA doses using weekly average doses (in mg/kg-day) as reported in Table 10 of the Supplemental Materials to {Ema, 2008, 787657@@author-year}.

BMR = benchmark response; ER = extra risk; PND = postnatal day; RD = relative deviation; SD = standard deviation; T4 = thyroxine; TWA = time-weighted average

3.2. DOSE-RESPONSE MODELING FOR NONCANCER ENDPOINTS

3.2.1. Evaluation of Model Fit

For each dichotomous endpoint where only summary data (i.e., number affected and total number exposed per group) were available, BMDS dichotomous models[1] were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test ($\chi 2$ p-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the benchmark response (BMR).

For each dichotomous endpoint for which incidence data were available for individual animals, BMDS nested dichotomous models[2] were fitted to the data using the maximum likelihood method. Each nested model was tested for goodness-of-fit using a bootstrap approach. Chi-square statistics were computed with both bootstrap iterations and original data. The p-value was the proportion of chi-square values from the iterations that were greater than the original chi-square value ($\chi 2$ p-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

For each continuous endpoint, BMDS continuous models[3] were fitted to the data using the maximum likelihood method. Model fit was assessed by a series of tests as follows. For each model, first the homogeneity of the variances was tested using a likelihood ratio test (BMDS Test 2). If Test 2 was not rejected ($\chi 2$ p-value ≥ 0.10), the model was fitted to the data assuming constant variance. If Test 2 was rejected ($\chi 2$ p-value < 0.10), the variance was modeled as a power function of the mean, and the variance model was tested for adequacy of fit using a likelihood ratio test (BMDS Test 3). For fitting models using either constant variance or modeled variance, models for the mean response were tested for adequacy of fit using a

[1]Unless otherwise specified, all available BMDS dichotomous models besides the alternative and nested dichotomous models were fitted. The following parameter restrictions were applied: for the LogLogistic model, restrict slope ≥1; for the Gamma and Weibull models, restrict power ≥1.

[2]Unless otherwise specified, all available BMDS nested dichotomous models were fitted. For the nested Logistic, NCTR, and Rai and van Ryzin models, power ≥1 was applied.

[3]Unless otherwise specified, all available BMDS continuous models were fitted. The following parameter restrictions were applied: for the polynomial models, restrict the coefficients b1 and higher to be nonnegative or nonpositive if the direction of the adverse effect is upward or downward, respectively; for the Hill, Power, and Exponential models, restrict power ≥1.

likelihood ratio test (BMDS Test 4, with $\chi 2$ p-value < 0.10 indicating inadequate fit). Other factors were also used to assess the model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

3.2.2. Model Selection

To select the appropriate model from which to derive the POD for each endpoint, the BMDL estimate (95% lower confidence limit on the benchmark dose [BMD], as estimated by the profile likelihood method) and Akaike's information criterion (AIC) value were used to select the model from among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close," that is, differed by at most 3-fold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

For nested dichotomous models, there are the options of including a litter-specific covariate and estimating intralitter correlations, yielding four combinations of option selections, as displayed in [REF _Ref532886487 \h]. All the three nested dichotomous models were fitted for every combination in the table, yielding four sets of models (12 model runs in total).

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. The combinations of option selections for the nested dichotomous models

Litter-specific covariates used Intralitter correlations estimated	Litter-specific covariates used Intralitter correlations assumed zero
Litter-specific covariates not used Intralitter correlations estimated	Litter-specific covariates not used Intralitter correlations assumed zero

The appropriate model was selected from this set of 12 models using the same procedure as for the non-nested models as described in Section 2.3.9 (page 39) of the Benchmark Dose Technical Guidance Document {U.S. EPA, 2012, 1239433}. If multiple litter specific covariates were tested, this same set of 12 modeling options was evaluated for each litter-specific covariate (e.g., litter size, implantation site, dam body weight) and the appropriate model was selected from the expanded set of modeling options (12 × number of litter-specific covariates considered) using the same procedure as for the non-nested models.

3.2.3. Modeling Results

Below are tables summarizing the modeling results for the noncancer endpoints modeled.

3

3.1

3.2

3.2.1

3.2.2

3.2.3

3.2.3.1 Thyroid

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for T4 in F0 parental male CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}; BMR = 10% RD from control mean, 15% RD from control mean, 20% RD from control mean, and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{15RD} (mg/kg-d)	$BMDL_{15RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.0473	33.926	259	177	399	274	Of the models without saturation that provided an adequate fit and a valid BMDL estimate, the Exponential 4 model with modeled variance was selected based on lowest AIC (BMDLs differed by <3).
Exponential (M4) **Exponential (M5)[c]**	**0.742**	**29.933**	**23.9**	**6.99**	**39.1**	**11.5**	
Hill	0.949	29.829	14.4	3.21	25.6	5.66	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.0418	34.174	303	227	455	341	
Model[a]	Goodness of fit		BMD_{20RD} (mg/kg-d)	$BMDL_{20RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.0473	33.926	548	376	866	511	
Exponential (M4) **Exponential (M5)[c]**	**0.742**	**29.933**	**57.9**	**17.2**	**101**	**29.5**	
Hill	0.949	29.829	42.0	9.11	94.9	Errorg	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.0418	34.174	607	454	906	595	

[a]Modeled variance case presented (BMDS Test 2 p-value = 0.0756, BMDS Test 3 p-value = 0.553), selected model in bold; scaled residuals for selected model for doses 0, 10.2, 101, and 1,008 mg/kg-day were -0.1665, 0.166, 0.03642, and -0.03619, respectively.

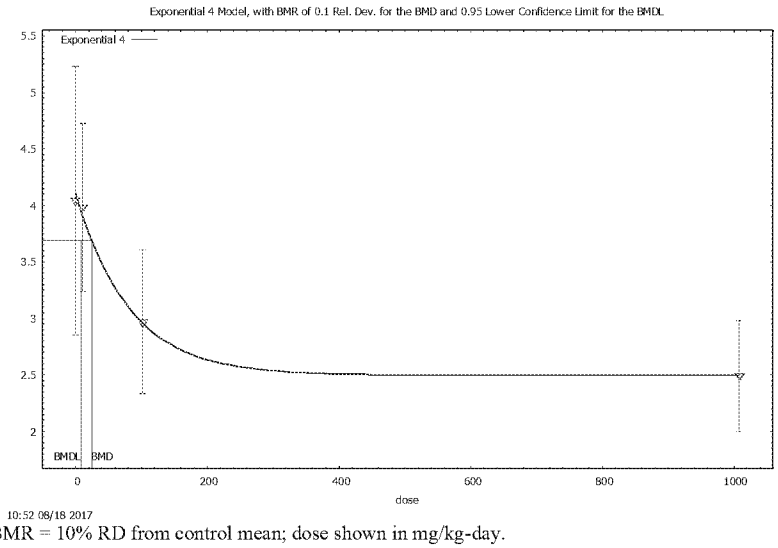
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

[c]For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.

[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.

[e]For the Polynomial 3° model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[g]BMD or BMDL computation failed for this model.

Data from {Ema, 2008, 787657@@author-year}



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is:
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
A modeled variance is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 23.8946
BMDL at the 95% confidence level = 6.99406

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284	-3.54227
rho	2.98463	2.72754

a	4.1075	4.242
b	0.0123219	0.00282274
d	1 (specified)	1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.56	-0.036

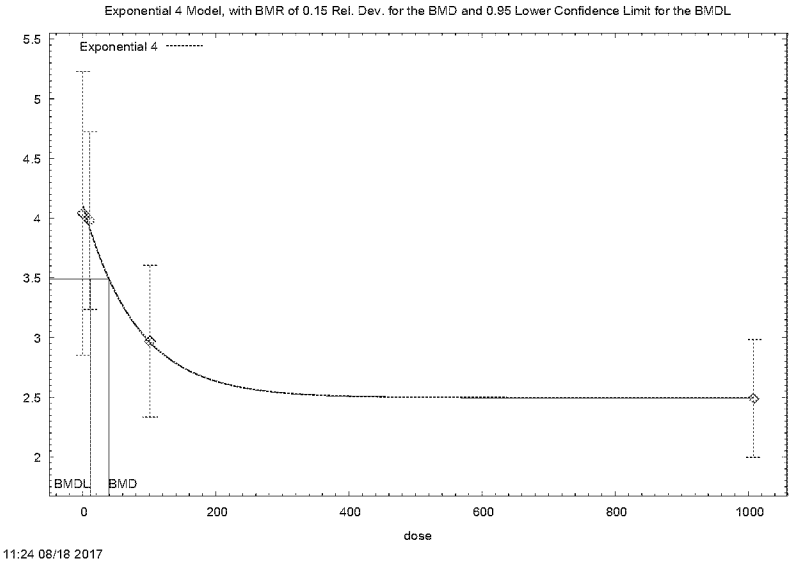
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

df = degree(s) of freedom



BMR = 15% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is:
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
A modeled variance is fit

Benchmark Dose Computation
BMR = 15% RD
BMD = 39.1317
BMDL at the 95% confidence level = 11.5235

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284	-3.54227
rho	2.98463	2.72754
a	4.1075	4.242
b	0.0123219	0.00282274
c	0.607906	0.55903

d	1 (specified)	1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.55	-0.036

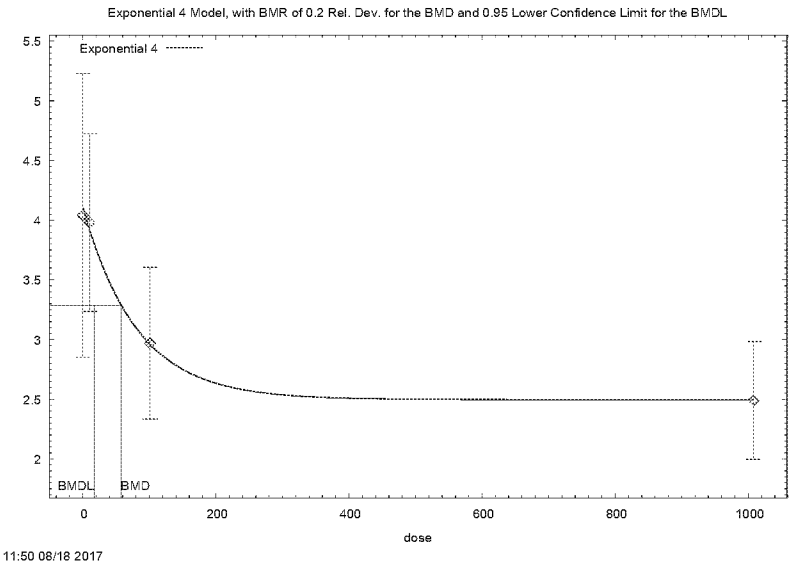
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

df = degree(s) of freedom



BMR = 20% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is:
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
A modeled variance is fit

Benchmark Dose Computation
BMR = 20% RD
BMD = 57.9065
BMDL at the 95% confidence level = 17.1892

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284	-3.54227
rho	2.98463	2.72754
a	4.1075	4.242
b	0.0123219	0.00282274
c	0.607906	0.55903
d	1 (specified)	1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.55	-0.036

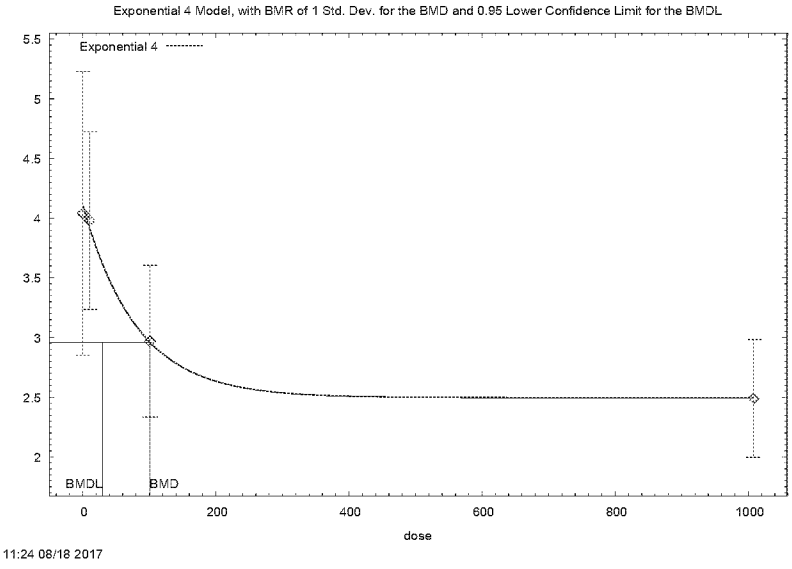
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

df = degree(s) of freedom



BMR = 1 SD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential 4 Model, for T4 in F0 parental CRL Sprague-Dawley male rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential 4 Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is:
Model 4: Y[dose] = a * [c-(c-1) * exp{-b * dose}]
A modeled variance is fit

Benchmark Dose Computation
BMR = 1 SD
BMD = 101.035
BMDL at the 95% confidence level = 29.4693

Parameter Estimates

Variable	Estimate	Default initial parameter values
lalpha	-3.94284	-3.54227
rho	2.98463	2.72754
a	4.1075	4.242
b	0.0123219	0.00282274
c	0.607906	0.55903
d	1 (specified)	1 (specified)

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	4.04	4.11	1.42	1.15	-0.167
10.2	8	3.98	3.92	0.89	1.07	0.166
101	8	2.97	2.961	0.76	0.71	0.036
1,008	8	2.49	2.50	0.59	0.55	-0.036

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	-12.76333	5	35.52665
A2	-9.319925	8	34.63985
A3	-9.91228	6	31.82456
fitted	-9.966286	5	29.93257
R	-19.64317	2	43.28634

Tests of Interest

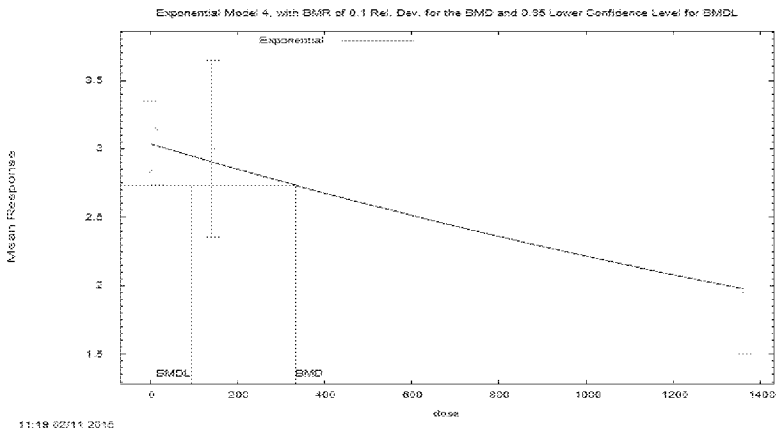
Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	20.65	6	0.002123
Test 2	6.887	3	0.07559
Test 3	1.185	2	0.553
Test 6a	0.108	1	0.7424

df = degree(s) of freedom

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}; BMR = 10% RD from control mean, 15% RD from control mean, 20% RD from control mean, and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{15RD} (mg/kg-d)	$BMDL_{15RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2)	0.479	3.7677	334	225	516	348	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M3)	0.298	5.3774	1,065	232	1,150	357	
Exponential (M4)	0.479	3.7677	334	93.8	516	154	
Exponential (M5)	N/A[b]	7.3774	1,086	103	1,158	143	
Hill	N/A[b]	7.3774	1,067	100	1,138	error[c]	
Power	0.298	5.3774	1,171	293	1,230	439	
Polynomial 3°	0.582	3.3778	902	816	1,032	934	
Polynomial 2°	0.580	3.3836	733	293	897	439	
Linear	0.505	3.6625	389	289	584	433	
Model[a]	Goodness of fit		BMD_{20RD} (mg/kg-d)	$BMDL_{20RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	
	p-value	AIC					
Exponential (M2)	0.479	3.7677	708	477	680	433	
Exponential (M3)	0.298	5.3774	1,240	491	1,234	446	
Exponential (M4)	0.479	3.7677	708	229	680	211	
Exponential (M5)	N/A[b]	7.3774	1,217	146	1,211	145	
Hill	N/A[b]	7.3774	1,185	error[c]	1,178	error[c]	
Power	0.298	5.3774	1,275	586	1,270	532	
Polynomial 3°	0.582	3.3778	1,136	1,028	1,126	999	
Polynomial 2°	0.580	3.3836	1,036	586	1,021	532	
Linear	0.505	3.6625	779	577	751	523	

[a]Constant variance case presented (BMDS Test 2 p-value = 0.579), selected model in bold; scaled residuals for selected model for doses 0, 14, 141.3, and 1,363 mg/kg-day were −0.9501, 0.5631, 0.4611, and −0.07911, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential Model 4, for T4 in F0 parental CRL Sprague-Dawley female rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 334.313
BMDL at the 95% confidence level = 93.781

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−1.06976	−1.11576
rho(S)	N/A	0
a	3.03677	3.297
b	0.000315155	0.00199958
c	0	0.566171
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.037	0.61	0.5857	−0.9501

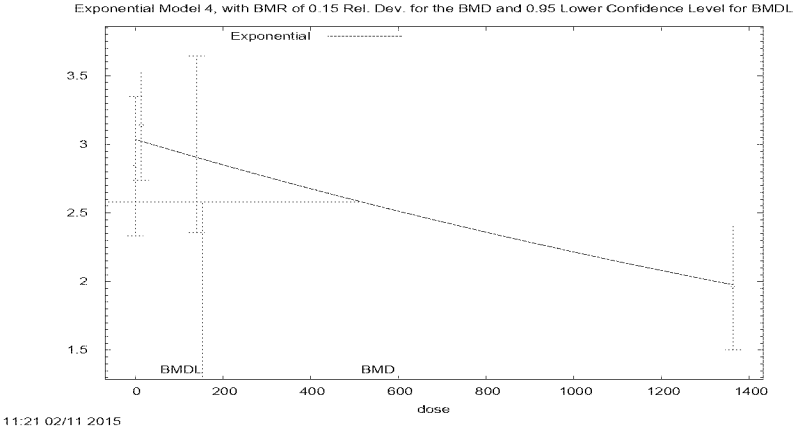
14	8	3.14	3.023	0.48	0.5857	0.5631
141.3	8	3	2.905	0.77	0.5857	0.4611
1,363	8	1.96	1.976	0.55	0.5857	−0.07911

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	−6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479



BMR = 15% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential Model 4, for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation
BMR = 15% RD
BMD = 515.679
BMDL at the 95% confidence level = 154.19

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−1.06976	−1.11576
rho(S)	N/A	0
a	3.03677	3.297
b	0.000315155	0.00199958
c	0	0.566171
d	1	1

Table of Data and Estimated Values of Interest

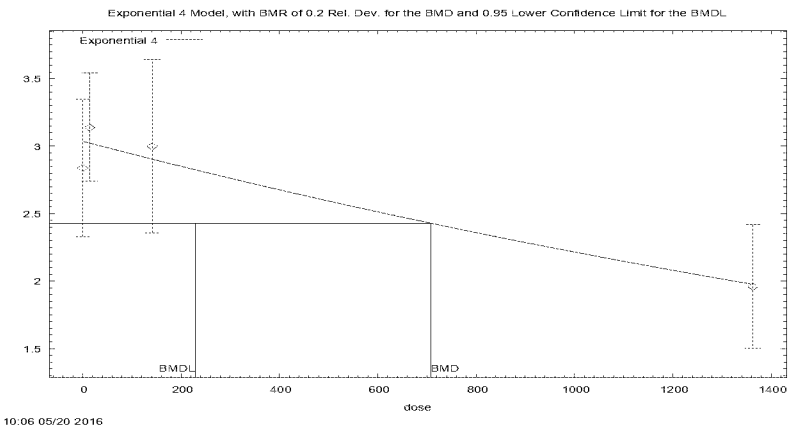
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.037	0.61	0.5857	−0.9501
14	8	3.14	3.023	0.48	0.5857	0.5631
141.3	8	3	2.905	0.77	0.5857	0.4611
1,363	8	1.96	1.976	0.55	0.5857	−0.07911

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	−6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479



BMR = 20% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 20% RD
BMD = 708.043
BMDL at the 95% confidence level = 228.829

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lnalpha	−1.06976	−1.11576
Rho	N/A	0
A	3.03677	3.297
B	0.000315155	0.00199958
C	0	0.566171
D	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.04	0.61	0.59	−0.9501

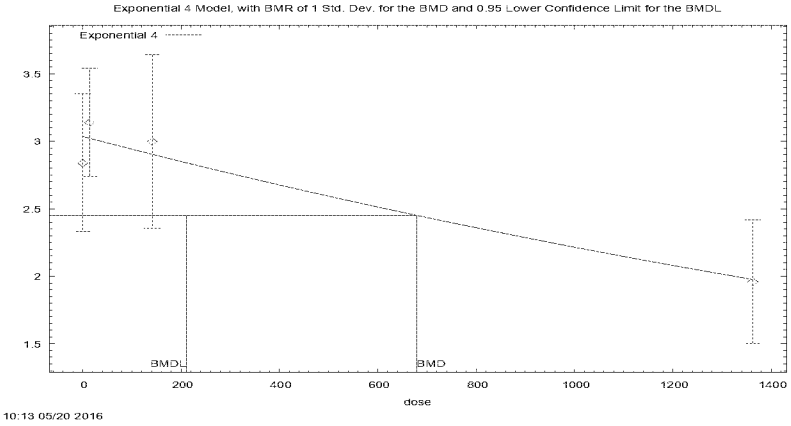
14	8	3.14	3.02	0.48	0.59	0.5631
141.3	8	3	2.9	0.77	0.59	0.4611
1,363	8	1.96	1.98	0.55	0.59	−0.07911

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	−6.115539	2	16.23108
4	1.116152	3	3.767695

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for T4 in F0 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 1.0000 Estimated SDs from control
BMD = 679.939
BMDL at the 95% confidence level = 210.769

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lnalpha	−1.06976	−1.11576
Rho	N/A	0
A	3.03677	3.297
B	0.000315155	0.00199958
C	0	0.566171
D	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	2.84	3.04	0.61	0.59	−0.9501
14	8	3.14	3.02	0.48	0.59	0.5631
141.3	8	3	2.9	0.77	0.59	0.4611
1,363	8	1.96	1.98	0.55	0.59	−0.07911

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	1.852186	5	6.295628
A2	2.83624	8	10.32752
A3	1.852186	5	6.295628
R	−6.115539	2	16.23108
4	1.116152	3	3.767695

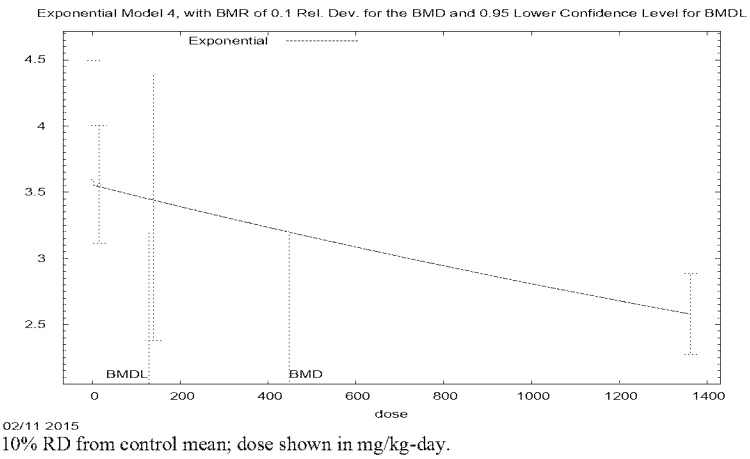
Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	17.9	6	0.006478
Test 2	1.968	3	0.5791
Test 3	1.968	3	0.5791
Test 6a	1.472	2	0.479

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}; BMR = 10% RD from control mean, 15% RD from control mean, 20% RD from control mean, and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{15RD} (mg/kg-d)	$BMDL_{15RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2)	0.305	19.978	448	320	691	493	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 (modeled variance) model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M3)	0.191	21.318	1,184	333	1,254	514	
Exponential (M4)	0.305	19.978	448	127	691	214	
Exponential (M5)	N/A[b]	23.318	1,193	153	1,259	144	
Hill	N/A[b]	23.318	1,131	153	1,204	error[c]	
Power	0.191	21.318	1,287	389	1,318	583	
Polynomial 3°	0.424	19.323	984	898	1,127	1,028	
Polynomial 2°	0.414	19.368	835	728	1,023	892	
Linear	0.323	19.868	498	379	747	568	
Model[a]	Goodness of fit		BMD_{20RD} (mg/kg-d)	$BMDL_{20RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	
	p-value	AIC					
Exponential (M2)	0.305	19.978	948	677	1,344	828	
Exponential (M3)	0.191	21.318	1,305	705	1,362	876	
Exponential (M4)	0.305	19.978	948	328	1,344	536	
Exponential (M5)	N/A[b]	23.318	1,309	148	1,362	152	
Hill	N/A[b]	23.318	1,269	error[c]	1,360	error[c]	
Power	0.191	21.318	1,341	777	1,363	932	
Polynomial 3°	0.424	19.323	1,240	1,132	1,360	1,193	
Polynomial 2°	0.414	19.368	1,181	1,030	1,357	1,115	
Linear	0.323	19.868	996	757	1,344	896	

[a]Modeled variance case presented (BMDS Test 2 p-value = 0.00445), selected model in bold; scaled residuals for selected model for doses 0, 14.3, 138.3, and 1,363 mg/kg-day were 0.105, 0.05257, −0.1637, and 0.008804, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential Model 4 (modeled variance) for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A modeled variance is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 447.782
BMDL at the 95% confidence level = 127.272

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−7.9144	−6.73265
rho	6.1823	5.13248
a	3.55422	3.7695
b	0.000235294	0.000283737
c	0	0.000684441
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.554	1.08	0.9635	0.105
14.3	8	3.56	3.542	0.53	0.9535	0.05257

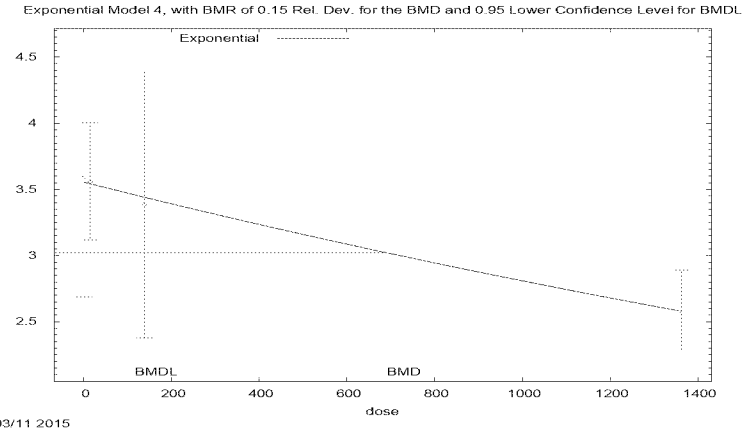
138.3	8	3.39	3.44	1.21	0.8713	−0.1637
1,363	8	2.58	2.579	0.37	0.3574	0.008804

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−9.516133	5	29.03227
A2	−2.971105	8	21.94221
A3	−4.802103	6	21.60421
R	−13.13332	2	30.26663
4	−5.988946	4	19.97789

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052



BMR = 15% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential Model 4, for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A modeled variance is fit

Benchmark Dose Computation
BMR = 15% RD
BMD = 690.705
BMDL at the 95% confidence level = 213.844

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lnalpha	−7.9144	−6.73265
Rho	6.1823	5.13248
A	3.55422	3.7695
B	0.000235294	0.000283737
C	0	0.000684441
D	1	1

Table of Data and Estimated Values of Interest

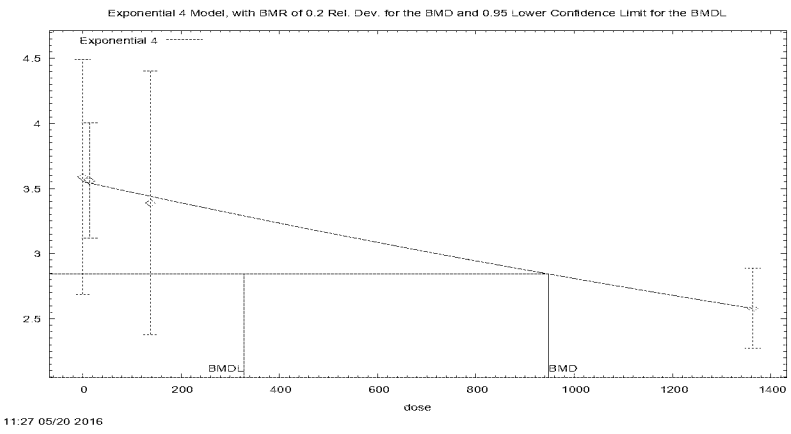
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.554	1.08	0.9635	0.105
14.3	8	3.56	3.542	0.53	0.9535	0.05257
138.3	8	3.39	3.44	1.21	0.8713	−0.1637
1,363	8	2.58	2.579	0.37	0.3574	0.008804

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−9.516133	5	29.03227
A2	−2.971105	8	21.94221
A3	−4.802103	6	21.60421
R	−13.13332	2	30.26663
4	−5.988946	4	19.97789

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052



BMR = 20% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with modeled variance for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A modeled variance is fit

Benchmark Dose Computation
BMR = 20% RD
BMD = 948.359
BMDL at the 95% confidence level = 328.063

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−7.9144	−6.73265
rho	6.1823	5.13248
a	3.55422	3.7695
b	0.000235294	0.000283737
c	0	0.000684441
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.55	1.08	0.96	0.105

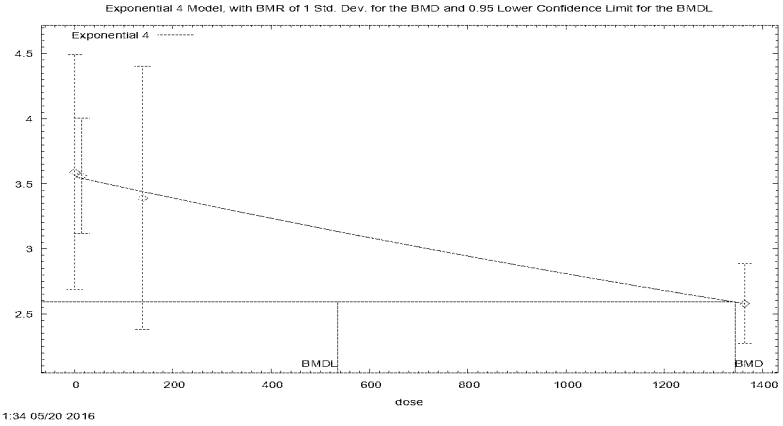
14.3	8	3.56	3.54	0.53	0.95	0.05257
138.3	8	3.39	3.44	1.21	0.87	−0.1637
1,363	8	2.58	2.58	0.37	0.36	0.008804

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−9.516133	5	29.03227
A2	−2.971105	8	21.94221
A3	−4.802103	6	21.60421
R	−13.13332	2	30.26663
4	−5.988946	4	19.97789

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with modeled variance for T4 in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A modeled variance is fit

Benchmark Dose Computation
BMR = 1.0000 Estimated SDs from control
BMD = 1,343.81
BMDL at the 95% confidence level = 536.006

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−7.9144	−6.73265
rho	6.1823	5.13248
a	3.55422	3.7695
b	0.000235294	0.000283737
c	0	0.000684441
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	8	3.59	3.55	1.08	0.96	0.105
14.3	8	3.56	3.54	0.53	0.95	0.05257
138.3	8	3.39	3.44	1.21	0.87	−0.1637
1,363	8	2.58	2.58	0.37	0.36	0.008804

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−9.516133	5	29.03227
A2	−2.971105	8	21.94221
A3	−4.802103	6	21.60421
R	−13.13332	2	30.26663
4	−5.988946	4	19.97789

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	20.32	6	0.002424
Test 2	13.09	3	0.004446
Test 3	3.662	2	0.1603
Test 6a	2.374	2	0.3052

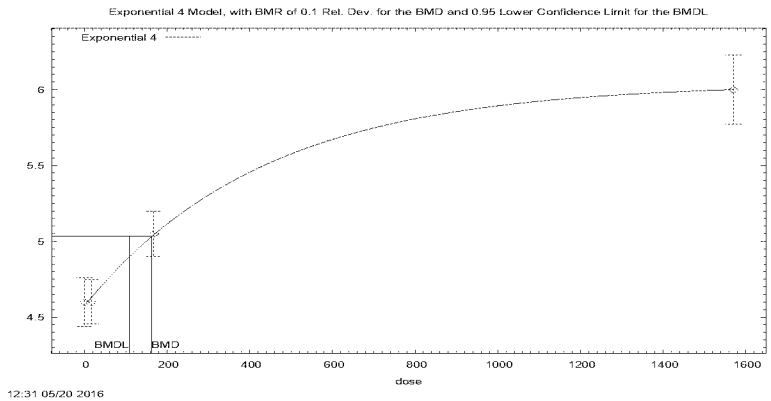
3.2.3.2 Liver

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in male F1 CRL rats exposed to HBCD on GD 0–PND 26, dose TWA gestation through lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.00369	−70.405	599	533	488	417	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest AIC and visual fit.
Exponential (M4)	**0.606**	**−79.345**	**163**	**109**	**120**	**80.5**	
Exponential (M5)	N/A[c]	−77.611	169	111	157	82.0	
Hill	N/A[c]	−77.611	169	104	156	75.4	
Powerd Polynomial 3°[e] Polynomial 2°[f] Linear	0.00590	−71.344	548	480	440	371	

[a]Constant variance case presented (BMDS Test 2 p-value = 0.462), selected model in bold; scaled residuals for selected model for doses 0, 16.5, 168, and 1,570 mg/kg-day were 0.3267, −0.3947, 0.05759, and −0.003788, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation through lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 162.81
BMDL at the 95% confidence level = 108.569

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.07833	−2.08162
rho	N/A	0
a	4.5759	4.37
b	0.00230233	0.00120199
c	1.3199	1.44165
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.6	4.576	0.37	0.3538	0.3267
16.5	21	4.6	4.63	0.32	0.3538	−0.3947

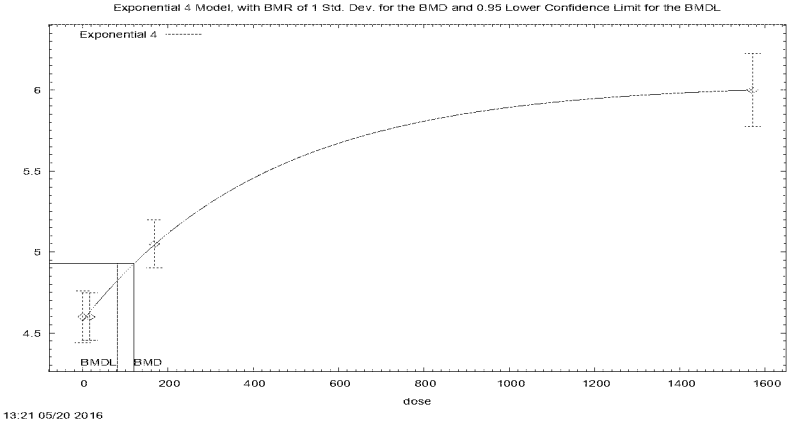
168	20	5.05	5.045	0.32	0.3538	0.05759
1,570	17	6	6	0.44	0.3538	−0.003788

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	43.80548	5	−77.61096
A2	45.09301	8	−74.18602
A3	43.80548	5	−77.61096
R	−5.569318	2	15.13864
4	43.67234	4	−79.34469

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	101.3	6	<0.0001
Test 2	2.575	3	0.4619
Test 3	2.575	3	0.4619
Test 6a	0.2663	1	0.6058



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation through lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 1.0000 Estimated SDs from control
BMD = 120.152
BMDL at the 95% confidence level = 80.5016

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.07833	−2.08162
rho	N/A	0
a	4.5759	4.37
b	0.00230233	0.00120199
c	1.3199	1.44165
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.6	4.576	0.37	0.3538	0.3267
16.5	21	4.6	4.63	0.32	0.3538	−0.3947
168	20	5.05	5.045	0.32	0.3538	0.05759
1,570	17	6	6	0.44	0.3538	−0.003788

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	43.80548	5	−77.61096
A2	45.09301	8	−74.18602
A3	43.80548	5	−77.61096
R	−5.569318	2	15.13864
4	43.67234	4	−79.34469

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	101.3	6	<0.0001
Test 2	2.575	3	0.4619
Test 3	2.575	3	0.4619
Test 6a	0.2663	1	0.6058

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F1 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA of gestation and lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.00217	−82.410	560	503	418	359	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest AIC.
Exponential (M4)	0.731	−92.555	165	115	109	75.8	
Exponential (M5)	N/A[c]	−90.673	170	116	126	76.4	
Hill	N/A[c]	−90.673	170	110	124	70.8	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear[g]	0.00403	−83.646	507	449	371	315	

[a]Constant variance case presented (BMDS Test 2 p-value = 0.711), selected model in bold; scaled residuals for selected model for doses 0, 16.5, 168, and 1,570 mg/kg-day were 0.2185, −0.263, 0.03719, and −0.002332, respectively.

[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.

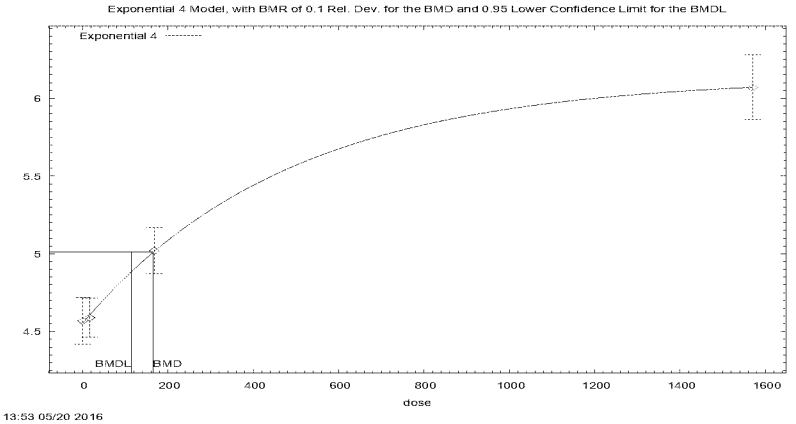
[c]No available degrees of freedom to calculate a goodness-of-fit value.

[d]The Power model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.

[e]For the Polynomial 3° model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model.

[f]The Polynomial 2° model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.

[g]The Linear model may appear equivalent to the Power model; however, differences exist in digits not displayed in the table. This also applies to the Polynomial 3° and Polynomial 2° models.



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling female CRL Sprague-Dawley rats exposed to HBCD GD 0–PND 26, dose TWA of gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 165.267
BMDL at the 95% confidence level = 114.71

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.28916	−2.29068
rho	N/A	0
a	4.5555	4.3415
b	0.00206359	0.00122548
c	1.34605	1.46804
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.57	4.555	0.35	0.3184	0.2185
16.5	21	4.59	4.608	0.28	0.3184	−0.263
168	20	5.02	5.017	0.32	0.3184	0.03719
1,570	14	6.07	6.07	0.36	0.3184	−0.002332

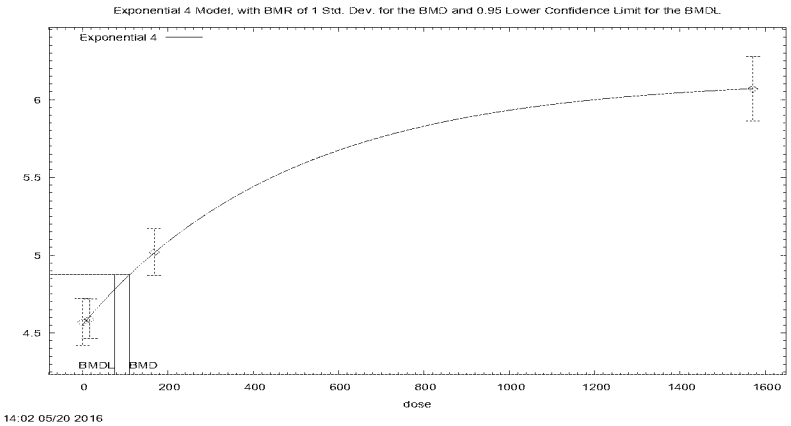
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.33659	5	−90.67319
A2	51.02517	8	−86.05034
A3	50.33659	5	−90.67319
R	−3.746671	2	11.49334
4	50.2774	4	−92.55481

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value

Test 1	109.5	6	<0.0001
Test 2	1.377	3	0.7109
Test 3	1.377	3	0.7109
Test 6a	0.1184	1	0.7308



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F1 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0−PND 26, dose TWA of gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 1.0000 Estimated SDs from control
BMD = 109.314
BMDL at the 95% confidence level = 75.8445

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.28916	−2.29068
rho	N/A	0
a	4.5555	4.3415
b	0.00206359	0.00122548
c	1.34605	1.46804

d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	23	4.57	4.555	0.35	0.3184	0.2185
16.5	21	4.59	4.608	0.28	0.3184	−0.263
168	20	5.02	5.017	0.32	0.3184	0.03719
1,570	14	6.07	6.07	0.36	0.3184	−0.002332

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.33659	5	−90.67319
A2	51.02517	8	−86.05034
A3	50.33659	5	−90.67319
R	−3.746671	2	11.49334
4	50.2774	4	−92.55481

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	109.5	6	<0.0001
Test 2	1.377	3	0.7109
Test 3	1.377	3	0.7109
Test 6a	0.1184	1	0.7308

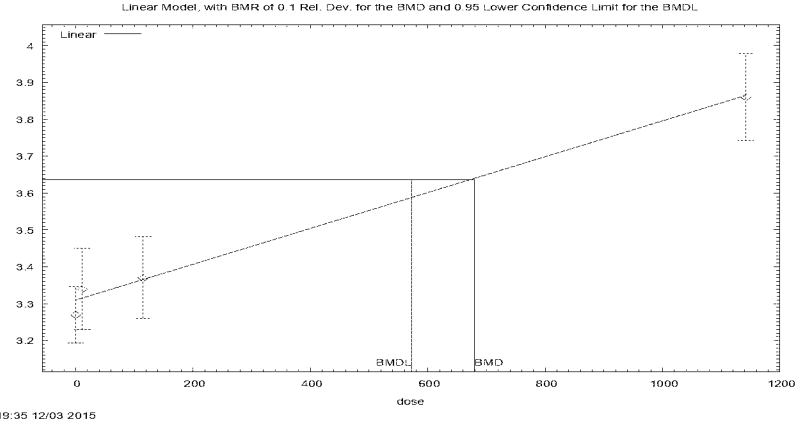
Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F1 adult male CRL Sprague-Dawley rats exposed to HBCD by diet for 15 weeks {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean.

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.626	−167.34	703	601	519	433	Of the models that provided an adequate fit and a valid BMDL estimate, the Linear constant variance model was selected based on lowest AIC (BMDLs differed by <3). Exponential M5
Exponential (M4)	0.366	−165.46	578	243	402	161	
Exponential (M5)	0.366	−165.46	578	121	402	118	
Hill	0.367	−165.46	582	error[c]	404	164	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.638	−167.38	680	573	496	409	

							and Hill models were excluded because both were saturated models in this case.

[a]Constant variance case presented (BMDS Test 2 p-value = 0.181), selected model in bold; scaled residuals for selected model for doses 0, 11.4, 115, and 1,142 mg/kg-day were -0.723, 0.587, 0.165, and -0.0218, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]BMD or BMDL computation failed for this model.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Polynomial 2° model. For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Linear model with constant variance for relative liver weight (g/100 g BW) in F1 adult male CRL Sprague-Dawley rats exposed to HBCD by diet for 15 weeks {Ema, 2008, 787657}.

Polynomial Model. (Version: 2.20; Date: 10/22/2014)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose
A constant variance model is fit

Benchmark Dose Computation.

BMR = 10% Relative deviation
BMD = 679.573
BMDL at the 95% confidence level = 572.977

Parameter Estimates

Variable	Estimate	Default Initial Parameter Values
alpha	0.0581671	0.0601744
rho	n/a	0
beta_0	3.30558	3.30581
beta_1	0.00048642	0.000486264

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Resid
0	24	3.27	3.31	0.18	0.241	-0.723
11.4	24	3.34	3.31	0.26	0.241	0.587
115	22	3.37	3.36	0.25	0.241	0.165
1142	24	3.86	3.86	0.28	0.241	-0.0218

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	87.137654	5	-164.275308
A2	89.578448	8	-163.156897
A3	87.137654	5	-164.275308
fitted	86.688502	3	-167.377004
R	55.373159	2	-106.746318

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	68.4106	6	<0.0001
Test 2	4.88159	3	0.1807
Test 3	4.88159	3	0.1807
Test 4	0.898304	2	0.6382

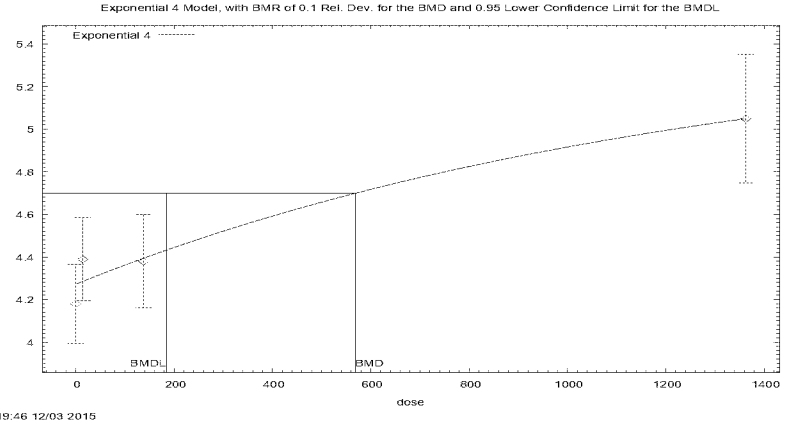
Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100g bw) in F1 adult female CRL Sprague-Dawley rats exposed to HBCD by diet for 17 weeks {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit					

	p-value	AIC	BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
Exponential (M2) Exponential (M3)[b]	0.311	−40.783	791	615	824	635	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3). Hill model was excluded because it was a saturated model in this case.
Exponential (M4) Exponential (M5)[c]	0.139	−38.934	569	184	603	203	
Hill	0.139	−38.937	575	186	610	208	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.316	−40.816	761	578	795	598	

[a]Constant variance case presented (BMDS Test 2 p-value = 0.917), selected model in bold; scaled residuals for selected model for doses 0, 14.3, 138, and 1,363 mg/kg-d were −0.9658, 1.098, −0.1406, and 0.002993, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]The Exponential (M5) model may appear equivalent to the Exponential (M4) model; however, differences exist in digits not displayed in the table.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver

weight (g/100 g BW) in F1 adult female CRL Sprague-Dawley rats exposed to HBCD by diet for 17 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 568.784
BMDL at the 95% confidence level = 184.198

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−1.60953	−1.63795
rho	N/A	0
a	4.27208	3.971
b	0.000792725	0.0012372
c	1.27553	1.33531
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.18	4.272	0.42	0.4472	−0.9658
14.3	22	4.39	4.285	0.44	0.4472	1.098
138	20	4.38	4.394	0.47	0.4472	−0.1406
1,363	13	5.05	5.05	0.5	0.4472	0.002993

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	24.56111	5	−39.12222
A2	24.8146	8	−33.6292
A3	24.56111	5	−39.12222
R	10.7627	2	−17.5254
4	23.46704	4	−38.93407

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	28.1	6	<0.0001
Test 2	0.507	3	0.9174

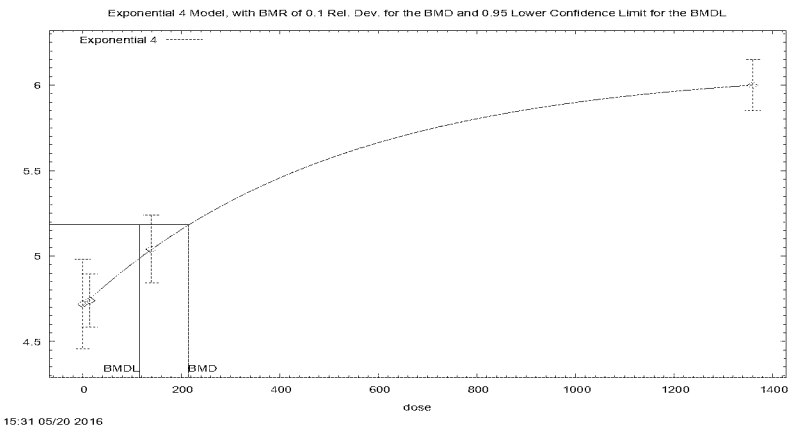
Test 3	0.507	3	0.9174
Test 6a	2.188	1	0.1391

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.235	−45.537	563	482	587	488	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M4)	0.882	−46.411	215	116	227	125	
Exponential (M5)	N/A[c]	−44.433	200	116	218	125	
Hill	N/A[c]	−44.433	207	112	223	120	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.278	−45.874	522	438	540	441	

[a]Constant variance case presented. Both constant variance assumption and modeled variance were not appropriate in this case: BMDS Tests 2 and 3 with constatnt variance assumption rejected the null hypothesis with p-value = 0.00438; Test 3 of modeled variance also rejected the null hypothesis. A sensitivity analysis (see below) indicated limited effect of variance on model fitting. Selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.09694, −0.1119, 0.01719, and −0.0007502, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 214.961
BMDL at the 95% confidence level = 115.944

Parameter Estimates

Variable	Estimate	Default initial parameter values
Lnalpha	−1.72548	−1.72578
Rho	N/A	0
A	4.71128	4.484
B	0.00192508	0.00133871
C	1.29509	1.405
D	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.711	0.59	0.422	0.09694

14.7	22	4.74	4.75	0.35	0.422	−0.1119
139.3	18	5.04	5.038	0.4	0.422	0.01719
1,360	13	6	6	0.25	0.422	−0.0007502

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	27.21664	5	−44.43327
A2	33.77721	8	−51.55442
A3	27.21664	5	−44.43327
R	−2.570126	2	9.140253
4	27.20553	4	−46.41105

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	72.69	6	<0.0001
Test 2	13.12	3	0.004382
Test 3	13.12	3	0.004382
Test 6a	0.02222	1	0.8815

Sensitivity analysis:
The fit to the means was adequate for Exponential M4 with constant variance, and their scaled residuals were small. However, Tests 2 and 3 rejected the null hypothesis with both constant variance assumption and modeled variance, indicating lack of fit to variances whether the variance was constant or modeled as a power of the means. To determine how much BMDL10%RD (116 mg/kg-day) was affected by the variance used, a sensitivity analysis was performed with constant variance by setting the standard deviation for all dose groups to the minimum or maximum observed values (0.25 and 0.59). Because the means were not changed and the constant-variance option was used, the parameters (including BMD) were unchanged. BMDLs (low confidence limit of BMD, BMR = 10% RD) were 147 mg/kg-day (with minimum standard deviation) and 96.7 mg/kg-day (with maximum standard deviation); the BMDLs were within twofold, suggesting limited effect of variance in this case. Therefore, the M4 model with constant variance was used to derive the BMD and BMDL for this data set.

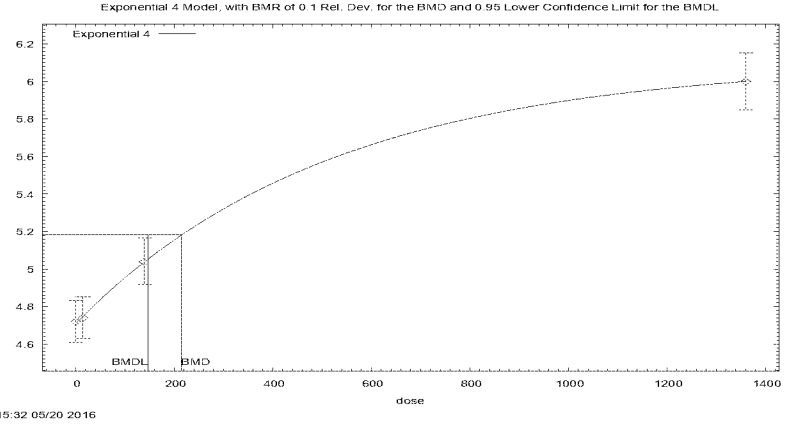
Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Sensitivity analysis with minimum SD as variance: Summary of BMD modeling results for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0−PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2)	0.0150	−122.66	563	512	

Exponential (M3)[b]					
Exponential (M4)	0.796	−128.99	215	147	
Exponential (M5)	N/A[c]	−127.05	200	147	
Hill	N/A[c]	−127.05	207	148	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.0241	−123.60	522	468	

[a]Constant variance case presented (BMDS Test 2 p-value = 1.000), selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.1681, −0.1941, 0.02981, and −0.001301, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD during gestation and lactation on GD 0−PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 214.961
BMDL at the 95% confidence level = 146.85

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.82651	−2.8274
rho	N/A	0
a	4.71128	4.484
b	0.00192508	0.00133871
c	1.29509	1.405
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.711	0.25	0.2434	0.1681
14.7	22	4.74	4.75	0.25	0.2434	−0.1941
139.3	18	5.04	5.038	0.25	0.2434	0.02981
1,360	13	6	6	0.25	0.2434	−0.001301

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	68.52739	5	−127.0548
A2	68.53022	8	−121.0604
A3	68.52739	5	−127.0548
R	10.89708	2	−17.79415
4	68.49396	4	−128.9879

Tests of Interest

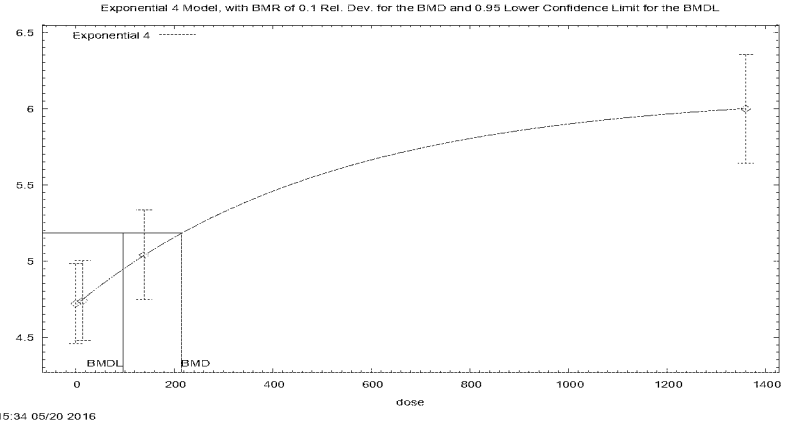
Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	115.3	6	<0.0001
Test 2	0.00567	3	0.9999
Test 3	0.00567	3	0.9999
Test 6a	0.06685	1	0.796

Table D-[STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Sensitivity analysis with maximum SD as variance: Summary of BMD modeling results for relative liver weight (g/10 0g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD by gestation and lactation on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Exponential (M2) Exponential (M3)[b]	0.454	−0.67698	563	459	
Exponential (M4)	0.913	−0.24352	215	96.7	
Exponential (M5)	N/A[c]	1.7445	200	96.9	
Hill	N/A[c]	1.7445	207	90.2	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	0.498	−0.86210	522	414	

[a]Constant variance case presented (BMDS Test 2 p-value = 1.000), selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.07126, −0.08227, 0.01264, and −0.0005523, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 214.962
BMDL at the 95% confidence level = 96.7112

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−1.10991	−1.11007
rho	N/A	0
a	4.71128	4.484
b	0.00192507	0.00133871
c	1.29509	1.405
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.711	0.59	0.5741	0.07126
14.7	22	4.74	4.75	0.59	0.5741	−0.08227
139.3	18	5.04	5.038	0.59	0.5741	0.01264
1,360	13	6	6	0.59	0.5741	−0.0005523

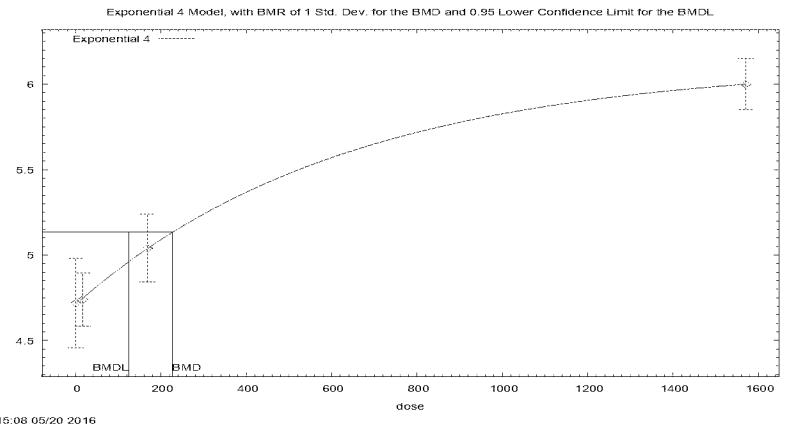
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	4.127765	5	1.744471
A2	4.130599	8	7.738801
A3	4.127765	5	1.744471
R	−14.77144	2	33.54287
4	4.121761	4	−0.2435229

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value

Test 1	37.8	6	<0.0001
Test 2	0.00567	3	0.9999
Test 3	0.00567	3	0.9999
Test 6a	0.01201	1	0.9127



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling male CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose TWA gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 1.0000 Estimated SDs from control
BMD = 227.183
BMDL at the 95% confidence level = 124.503

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−1.72556	−1.72578
rho	N/A	0
a	4.71255	4.484
b	0.00156899	0.00115941

c	1.29864	1.405
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	4.72	4.713	0.59	0.422	0.08283
16.5	22	4.74	4.749	0.35	0.422	−0.09464
168	18	5.04	5.039	0.4	0.422	0.01356
1,570	13	6	6	0.25	0.422	−0.0006035

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	27.21664	5	−44.43327
A2	33.77721	8	−51.55442
A3	27.21664	5	−44.43327
R	−2.570126	2	9.140253
4	27.20864	4	−46.41727

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	72.69	6	<0.0001
Test 2	13.12	3	0.004382
Test 3	13.12	3	0.004382
Test 6a	0.016	1	0.8993

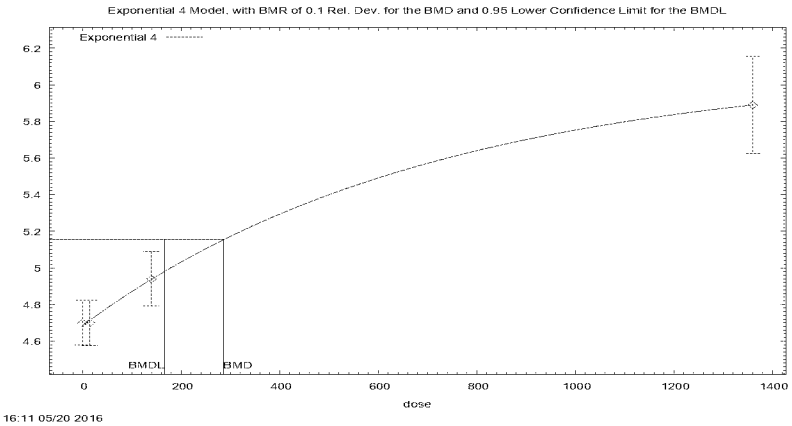
Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in F2 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0–PND 26, dose as TWA of gestation and lactation {Ema, 2008, 787657}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2) Exponential (M3)[b]	0.265	−92.639	589	520	400	339	Of the models that provided an adequate fit and a valid BMDL estimate, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M4)	0.759	−93.205	286	166	177	103	
Exponential (M5)	N/A[c]	−91.299	168	141	149	104	
Hill	N/A[c]	−91.299	153	error[d]	144	101	
Power[e] Polynomial 3°[f] Polynomial 2°[g]	0.323	−93.039	549	477	367	307	

Linear							

[a]Constant variance case presented (BMDS Test 2 p-value = 0.192), selected model in bold; scaled residuals for selected model for doses 0, 14.7, 139.3, and 1,360 mg/kg-day were 0.2031, −0.2277, 0.03152, and −0.001049, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]BMD or BMDL computation failed for this model.
[e]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[f]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[g]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {Ema, 2008, 787657@@author-year}



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver

weight (g/100 g BW) in F2 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0−PND 26, dose as TWA of gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 286.259
BMDL at the 95% confidence level = 166.437

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.33164	−2.33288
rho	N/A	0
a	4.68619	4.465
b	0.00140932	0.00130926
c	1.30123	1.38511
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	4.7	4.686	0.27	0.3117	0.2031
14.7	22	4.7	4.715	0.28	0.3117	−0.2277
139.3	20	4.94	4.938	0.32	0.3117	0.03152
1,360	13	5.89	5.89	0.44	0.3117	−0.001049

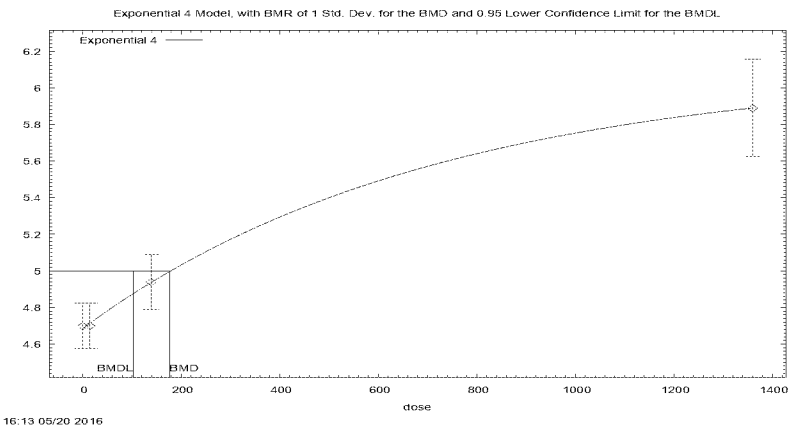
Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.6495	5	−91.299
A2	53.0199	8	−90.03981
A3	50.6495	5	−91.299
R	9.931909	2	−15.86382
4	50.60242	4	−93.20485

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	86.18	6	<0.0001
Test 2	4.741	3	0.1918

Test 3	4.741	3	0.1918
Test 6a	0.09415	1	0.759



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for relative liver weight (g/100 g BW) in F2 weanling female CRL Sprague-Dawley rats exposed to HBCD on GD 0−PND 26, dose as TWA of gestation and lactation {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 1.0000 Estimated SDs from control
BMD = 177.017
BMDL at the 95% confidence level = 102.961

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	−2.33164	−2.33288
rho	N/A	0
a	4.68619	4.465
b	0.00140932	0.00130926
c	1.30123	1.38511
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	4.7	4.686	0.27	0.3117	0.2031
14.7	22	4.7	4.715	0.28	0.3117	−0.2277
139.3	20	4.94	4.938	0.32	0.3117	0.03152
1,360	13	5.89	5.89	0.44	0.3117	−0.001049

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	50.6495	5	−91.299
A2	53.0199	8	−90.03981
A3	50.6495	5	−91.299
R	9.931909	2	−15.86382
4	50.60242	4	−93.20485

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	86.18	6	<0.0001
Test 2	4.741	3	0.1918
Test 3	4.741	3	0.1918
Test 6a	0.09415	1	0.759

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in male CRL Sprague-Dawley rats exposed to HBCD by gavage for 13 weeks {WIL Research, 2001, 787787}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Modeled with constant variance							No model showed adequate fit. Dropping highest dose is not expected to help in this case.
Exponential (M2) Exponential (M3)[b]	3.14 × 10−4	−67.830	328	283	269	219	
Exponential (M4)[c]	3.92 × 10−4	−69.396	164	97.7	128	77.9	
Exponential (M5)[d]	3.92 × 10−4	−69.396	164	97.7	128	77.9	
Hill	4.91 × 10−4	−69.815	145	74.8	113	59.7	

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Power[e] Polynomial 3°[f] Polynomial 2°[g] Linear	5.14 × 10−4	−68.817	290	244	234	187	
Modeled with modeled variance							
Exponential (M2) Exponential (M3)[b]	0.00119	−68.721	337	295	320	245	
Exponential (M4)[c]	5.50 × 10−4	−68.244	204	103	187	67.5	
Exponential (M5)[d]	5.50 × 10−4	−68.244	204	103	187	67.5	
Hill	5.84 × 10−4	−68.355	192	35.9	173	106	
Power[e] Polynomial 3°[f] Polynomial 2°[g] Linear	0.00161	−69.324	299	256	282	210	

[a]Constant variance (BMDS Test 2 p-value = 0.0644, BMDS Test 3 p-value = 0.0644) and nonconstant variance cases presented, no model was selected as a best-fitting model.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]The Exponential (M4) model may appear equivalent to the Exponential (M5) model; however, differences exist in digits not displayed in the table.
[d]The Exponential (M5) model may appear equivalent to the Exponential (M4) model; however, differences exist in digits not displayed in the table.
[e]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[f]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[g]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

Data from {WIL Research, 2001, 787787@@author-year}

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for relative liver weight (g/100 g BW) in female CRL Sprague-Dawley rats exposed to HBCD by gavage for 13 weeks {WIL Research, 2001, 787787}; BMR = 10% RD from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Modeled with constant variance							
Exponential (M2) Exponential (M3)[b]	<0.0001	−39.545	310	261	332	267	No model showed adequate fit. Dropping highest

Exponential (M4) Exponential (M5)[c]	2.59 × 10−4	−44.035	101	56.0	106	61.8	dose is not expected to help in this case
Hill	5.71 × 10−4	−45.515	69.3	30.6	73.3	34.6	
Power[d] Polynomial 3°[e] Polynomial 2°[f] Linear	<0.0001	−40.679	270	220	287	226	
Modeled with modeled variance							
Exponential (M2) Exponential (M3)[b]	<0.0001	−38.793	319	269	374	282	
Exponential (M4) Exponential (M5)[c]	1.72 × 10−4	−42.217	53.4	28.5	38.3	16.0	
Hill	0.00115	−45.763	39.2	20.7	26.0	11.6	
Powerd Polynomial 3°[e] Polynomial 2°[f] Linear	<0.0001	−39.727	278	227	327	237	

[a]Constant variance (BMDS Test 2 p-value = 0.461, BMDS Test 3 p-value = 0.461) and nonconstant variance presented; no model was selected as a best-fitting model.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]For the Exponential (M5) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M4) model.
[d]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[e]For the Polynomial 3° model, the b3 and b2 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.

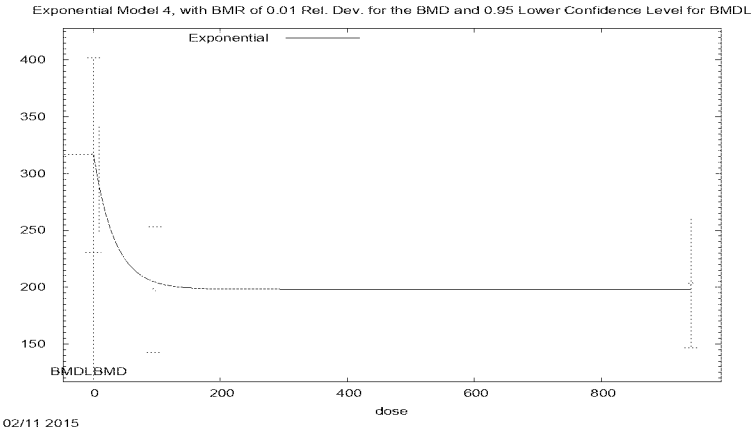
3.2.3.3 Reproductive

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}; BMR = 1% RD from control mean, 5% RD from control mean, and 10% RD from control mean

	Goodness of fit								
Modela	p-value	AIC	BMD_{1RD} (mg/kg-d)	$BMDL_{1RD}$ (mg/kg-d)	BMD_{5RD} (mg/kg-d)	$BMDL_{5RD}$ (mg/kg-d)	BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
Exponential (M2) Exponential (M3)[b]	0.0130	408.57	26.8	13.9	137	71.0	281	146	Exponential M4 constant variance selected as only
Exponential (M4)	0.688	402.05	0.883	0.252	4.67	1.33	10.1	2.87	

Exponential (M5)	N/A[c]	403.91	4.09	0.259	8.23	1.37	11.4	2.95	model with adequate fit.
Hill	N/A[c]	403.91	8.00	error[d]	9.28	1.10	9.99	2.50	
Power[e] Polynomial 2°[f] Linear Polynomial 3°[g]	0.0117	408.78	33.1	19.8	165	99.0	331	198	

[a]Constant variance case presented (BMDS Test 2 p-value = 0.242), selected model in bold; scaled residuals for selected model for doses 0, 9.6, 96.3, and 940.7 mg/kg-day were −0.129, 0.1915, −0.2611, and 0.1987, respectively.
[b]For the Exponential (M3) model, the estimate of d was 1 (boundary). The models in this row reduced to the Exponential (M2) model.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]BMD or BMDL computation failed for this model.
[e]For the Power model, the power parameter estimate was 1. The models in this row reduced to the Linear model.
[f]For the Polynomial 2° model, the b2 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Linear model.
[g]The Polynomial 3° model may appear equivalent to the Linear model; however, differences exist in digits not displayed in the table.



BMR = 1% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential M4, for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 1% RD
BMD = 0.883338

BMDL at the 95% confidence level = 0.251965

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	8.85121	8.84717
rho(S)	N/A	0
a	319.71	332.115
b	0.0301725	0.0026785
c	0.619779	0.567503
d	1	1

Table of Data and Estimated Values of Interest

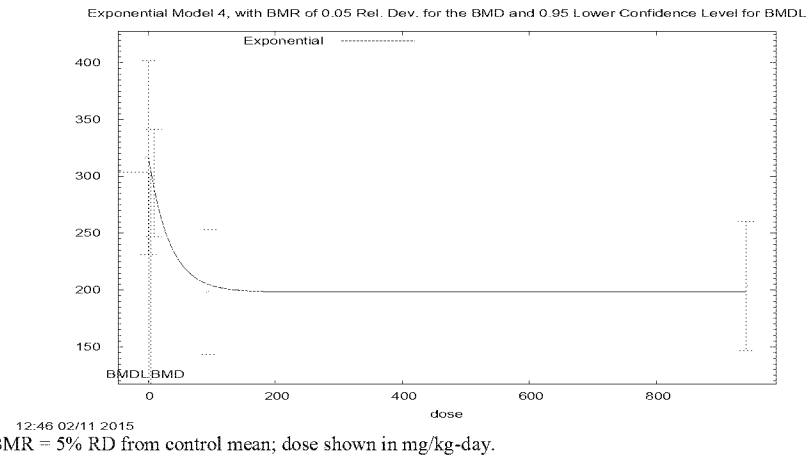
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	10	316.3	319.7	119.5	83.56	−0.129
9.6	10	294.2	289.1	66.3	83.56	0.1915
96.3	10	197.9	204.8	76.9	83.56	−0.2611
940.7	10	203.4	198.1	79.5	83.56	0.1987

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−196.9435	5	403.8869
A2	−194.8505	8	405.701
A3	−196.9435	5	403.8869
R	−203.7104	2	411.4207
4	−197.0241	4	402.0483

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	17.72	6	0.006972
Test 2	4.186	3	0.2421
Test 3	4.186	3	0.2421
Test 6a	0.1613	1	0.6879



BMR = 5% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential Model 4, for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 5% RD
BMD = 4.67281
BMDL at the 95% confidence level = 1.32975

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	8.85121	8.84717
rho(S)	N/A	0
a	319.71	332.115
b	0.0301725	0.0026785
c	0.619779	0.567503
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	10	316.3	319.7	119.5	83.56	−0.129
9.6	10	294.2	289.1	66.3	83.56	0.1915

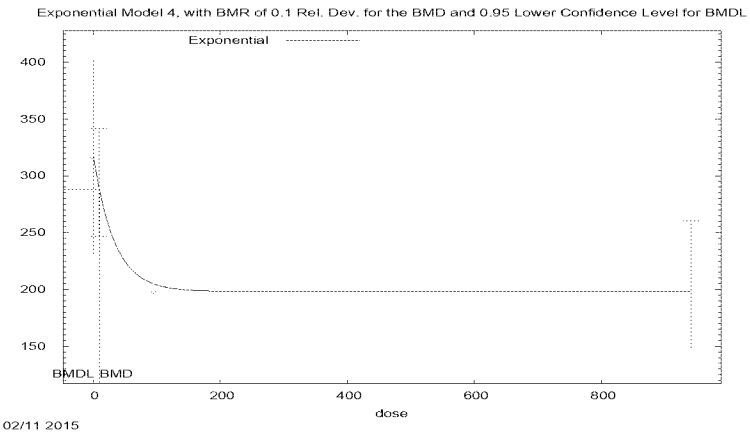
96.3	10	197.9	204.8	76.9	83.56	−0.2611
940.7	10	203.4	198.1	79.5	83.56	0.1987

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−196.9435	5	403.8869
A2	−194.8505	8	405.701
A3	−196.9435	5	403.8869
R	−203.7104	2	411.4207
4	−197.0241	4	402.0483

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	17.72	6	0.006972
Test 2	4.186	3	0.2421
Test 3	4.186	3	0.2421
Test 6a	0.1613	1	0.6879



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure D-[STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose, with fitted curve for Exponential M4, for primordial follicles in F1 parental female CRL Sprague-Dawley rats exposed to HBCD by diet for 18 weeks {Ema, 2008, 787657}.

Exponential Model (Version: 1.9; Date: 01/29/2013)

The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]

A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 10.1143
BMDL at the 95% confidence level = 2.86589

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	8.85121	8.84717
rho(S)	N/A	0
a	319.71	332.115
b	0.0301725	0.0026785
c	0.619779	0.567503
d	1	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	10	316.3	319.7	119.5	83.56	−0.129
9.6	10	294.2	289.1	66.3	83.56	0.1915
96.3	10	197.9	204.8	76.9	83.56	−0.2611
940.7	10	203.4	198.1	79.5	83.56	0.1987

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−196.9435	5	403.8869
A2	−194.8505	8	405.701
A3	−196.9435	5	403.8869
R	−203.7104	2	411.4207
4	−197.0241	4	402.0483

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	17.72	6	0.006972
Test 2	4.186	3	0.2421
Test 3	4.186	3	0.2421
Test 6a	0.1613	1	0.6879

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to

HBCD in diet for 14 weeks, TWA F0 and F1 premating dose {Ema, 2008, 787657}; BMR = 5% ER and 10% ER

Model[a]	Goodness of fit		BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Gamma Weibull Multistage 3° Multistage 2° Quantal-Linear	0.0881	120.47	617	263	1,266	541	No models provided an adequate fit and a valid BMDL estimate; therefore no model was selected.
Dichotomous-Hill	N/A[b]	119.61	15.1	error[c]	35.8	13.4	
Logistic	0.0806	120.75	824	482	1,401	817	
LogLogistic	0.0897	120.43	584	230	1,232	486	
Probit	0.0815	120.72	797	449	1,392	781	
LogProbit	0.396	118.31	6.18	error[c]	159	error[c]	

[a]No model was selected as a best-fitting model.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.

Data from {Ema, 2008, 787657@@author-year}

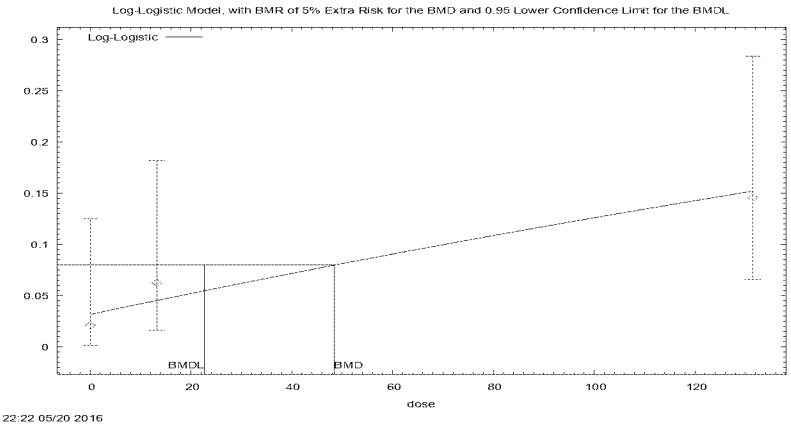
Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 premating dose, high dose dropped {Ema, 2008, 787657}; BMR = 5% ER and 10% ER.

Model[a]	Goodness of fit		BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Gamma[b]	0.457	76.591	51.1	25.6	105	52.5	Of the models that provided an adequate fit and a valid BMDL estimate, the LogLogistic model was selected based on lowest AIC.
Logistic	0.374	76.860	77.3	53.3	121	85.5	
LogLogistic	0.469	76.560	48.5	22.7	102	47.9	
Probit	0.382	76.832	73.6	49.3	120	81.1	
LogProbit	N/A[c]	78.045	18.0	error[d]	74.8	error[d]	
Weibull[e] Quantal-Linear[f]	0.457	76.591	51.1	25.6	105	52.5	
Multistage 2°[g]	0.457	76.591	51.1	25.6	105	52.5	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 13.3, and 131.5 mg/kg-day were −0.422, 0.575, and −0.128, respectively.
[b]The Gamma model may appear equivalent to the Weibull model; however, differences exist in digits not displayed in the table. This also applies to the Multistage 2° and Quantal-Linear models.
[c]No available degrees of freedom to calculate a goodness-of-fit value.
[d]BMD or BMDL computation failed for this model.
[e]For the Weibull model, the power parameter estimate was 1. The models in this row reduced to the Quantal-Linear model.

[f]The Quantal-Linear model may appear equivalent to the Gamma model; however, differences exist in digits not displayed in the table. This also applies to the Multistage 2° model.
[g]The Multistage 2° model may appear equivalent to the Gamma model; however, differences exist in digits not displayed in the table. This also applies to the Weibull and Quantal-Linear models.

Data from {Ema, 2008, 787657@@author-year}



BMR = 5% ER; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose with fitted curve for LogLogistic model for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 premating dose, high dose dropped {Ema, 2008, 787657}.

Logistic Model (Version: 2.14; Date: 2/28/2013)
The form of the probability function is: P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
Slope parameter is restricted as slope >= 1

Benchmark Dose Computation
BMR = 5% ER
BMD = 48.4809
BMDL at the 95% confidence level = 22.7093

Parameter Estimates

Variable	Estimate	Default initial parameter values
background	0.0314626	0.0208333
intercept	−6.8256E+00	−6.4682E+00
slope	1	1

Analysis of Deviance Table

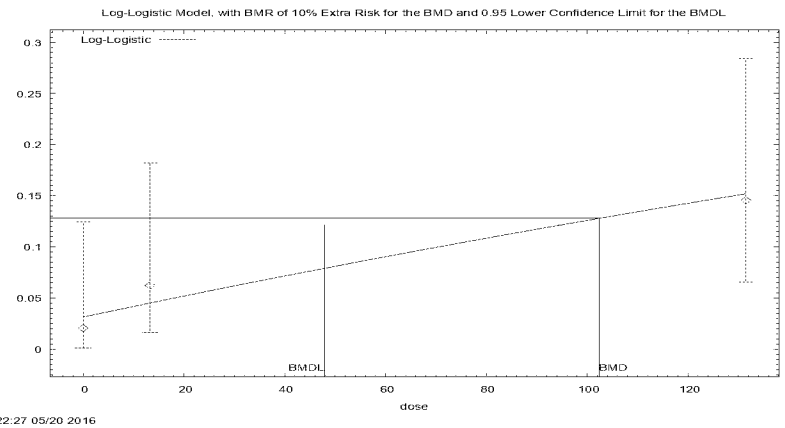
Model	Log (likelihood)	Number of parameters	Deviance	Test df	p-value
Full model	−36.0225	3			
Fitted model	−36.28	2	0.514904	1	0.473
Reduced model	−38.8598	1	5.6746	2	0.05858

AIC: = 76.56

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0.0315	1.51	1	48	−0.422
13.3	0.0452	2.172	3	48	0.575
131.5	0.1525	7.318	7	48	−0.128

Chi^2 = 0.52, df = 1, p-value = 0.4687



BMR = 10% ER; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose with fitted curve for LogLogistic model for incidence of non-pregnancy in F0 and F1 CRL female rats combined exposed to HBCD in diet for 14 weeks, TWA F0 and F1 premating dose, high dose dropped {Ema, 2008, 787657}.

Logistic Model (Version: 2.14; Date: 2/28/2013)
The form of the probability function is: P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
Slope parameter is restricted as slope >= 1

Benchmark Dose Computation
BMR = 10% ER
BMD = 102.349

BMDL at the 95% confidence level = 47.9419

Parameter Estimates

Variable	Estimate	Default initial parameter values
background	0.0314626	0.0208333
intercept	−6.8256E+00	−6.4682E+00
slope	1	1

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test df	p-value
Full model	−36.0225	3			
Fitted model	−36.28	2	0.514904	1	0.473
Reduced model	−38.8598	1	5.6746	2	0.05858

AIC: = 76.56

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0.0315	1.51	1	48	−0.422
13.3	0.0452	2.172	3	48	0.575
131.5	0.1525	7.318	7	48	−0.128

Chi^2 = 0.52, df = 1, p-value = 0.4687

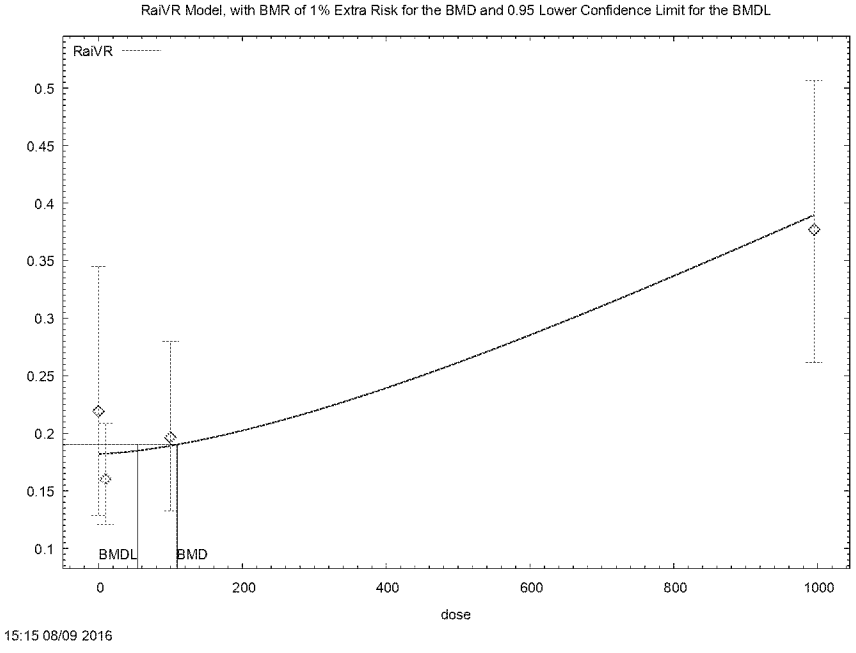
3.2.3.4 Developmental

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for offspring loss from implantation through PND 4 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams {Ema, 2008, 787657}; BMR = 1% ER and 5% ER

Model[a]	Goodness of Fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Litter-specific covariate = implantation size; intra-litter correlations estimated							Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the NCTR/Rai and Van Ryzin model (litter-specific covariate not used; intra-litter correlations estimated) was selected based on lowest BMDL
Nested Logistic	0.1776	1,236.98	523.682	17.8051	708.771	92.7735	
NCTR	0.1770	1,237.29	450.409	225.409	659.055	329.826	
Rai and Van Ryzin	0.1984	1,236.26	371.593	185.81	538.091	269.046	
Litter-specific covariate = implantation size; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	1,337.62	560.759	26.8162	740.805	139.727	
NCTR	0.0000	1,335.98	553.123	460.936	739.356	616.13	
Rai and Van Ryzin	0.0000	1,337.63	138.735	86.7096	291.342	291.342	
Litter-specific covariate not used; intra-litter correlations estimated							
Nested Logistic	0.1377	1,234.32	105.863	17.0526	301.093	88.853	

Model[a]	Goodness of Fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
NCTR[b] Rai and Van Ryzin	0.1423	1,234.32	108.957	54.4786	315.584	157.792	(BMDLs differed by >3).
Litter-specific covariate not used; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	1,336.56	132.255	25.2574	353.37	131.605	
NCTRb Rai and Van Ryzin	0.0000	1,336.56	136.105	68.0523	367.95	183.975	

[a]Because the individual animal data were available, the BMDS nested models were fitted, with the selected model in bold. For the selected model, the proportion of litters with scaled residuals above 2 in absolute value for doses 0, 9.7, 100, and 995 mg/kg-day were 2/23, 1/23, 1/20, and 1/21, respectively.
[b]With the litter-specific covariate not used, the NCTR and Rai and van Ryzin models yielded identical results.



BMR = 1% ER; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose, with fitted curve for the nested Rai and Van Ryzin model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from implantation through PND 4 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams {Ema, 2008, 787657}.

Rai and Van Ryzin Model (Version: 2.12; Date: 04/27/2015)
The form of the probability function is:
Prob. = [1-exp(-Alpha-Beta*Dose^Rho)]*exp(-(Th1+Th2*Dose)*Rij),
where Rij is the litter specific covariate.
Restrict Power rho >= 1.

Benchmark Dose Computation
To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.425287
BMR = 1% ER
BMD = 108.957
BMDL at the 95% confidence level = 54.4787

Parameter Estimates

Variable	Estimate	(Default) Initial Parameter Values
alpha	0.201085	0.201085
beta	7.58104 × 10−6	7.58104 × 10−6
rho	1.53267	1.53267
phi1	0.222343	0.222343
phi2	0.0213907	0.0213907
phi3	0.0759418	0.0759418
phi4	0.277171	0.277171

Log-likelihood: −610.162 AIC: 1,234.32

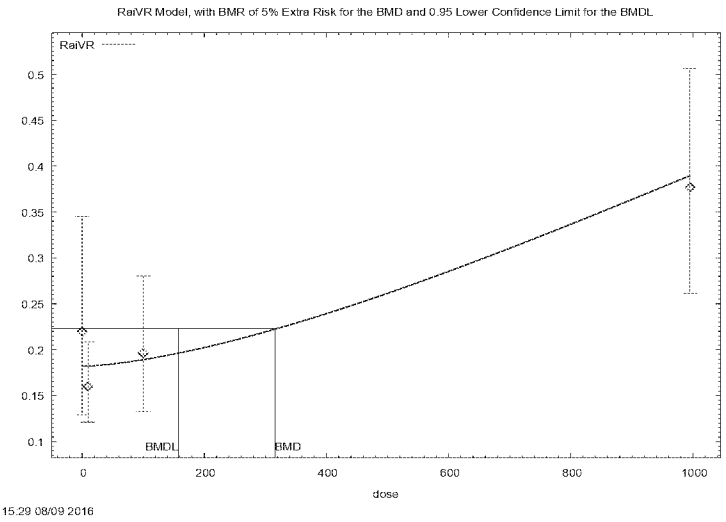
Goodness-of-Fit Table

Dose	Lit.-Spec. Cov.	Litter Est. Prob.	Litter Size	Scaled Expected	Scaled Observed	Scaled Residual
0.0000	9.0000	0.182	9	1.639	3	0.7049
0.0000	10.0000	0.182	10	1.822	4	1.0303
0.0000	11.0000	0.182	11	2.004	5	1.3037
0.0000	11.0000	0.182	11	2.004	0	−0.8718
0.0000	12.0000	0.182	12	2.186	1	−0.4778
0.0000	13.0000	0.182	13	2.368	0	−0.8885
0.0000	13.0000	0.182	13	2.368	3	0.2371
0.0000	13.0000	0.182	13	2.368	3	0.2371
0.0000	13.0000	0.182	13	2.368	0	−0.8885
0.0000	14.0000	0.182	14	2.550	1	−0.5442
0.0000	14.0000	0.182	14	2.550	3	0.1579
0.0000	15.0000	0.182	15	2.732	15	4.0466
0.0000	15.0000	0.182	15	2.732	11	2.7271
0.0000	16.0000	0.182	16	2.915	4	0.3377
0.0000	16.0000	0.182	16	2.915	2	−0.2845
0.0000	16.0000	0.182	16	2.915	2	−0.2845
0.0000	16.0000	0.182	16	2.915	1	−0.5956
0.0000	16.0000	0.182	16	2.915	2	−0.2845
0.0000	16.0000	0.182	16	2.915	2	−0.2845
0.0000	17.0000	0.182	17	3.097	3	−0.0285

0.0000	17.0000	0.182	17	3.097	0	−0.9115
0.0000	17.0000	0.182	17	3.097	6	0.8546
0.0000	18.0000	0.182	18	3.279	1	−0.6365
9.7000	2.0000	0.182	2	0.365	2	2.9630
9.7000	12.0000	0.182	12	2.188	5	1.8912
9.7000	13.0000	0.182	13	2.371	3	0.4032
9.7000	13.0000	0.182	13	2.371	0	−1.5189
9.7000	13.0000	0.182	13	2.371	4	1.0439
9.7000	14.0000	0.182	14	2.553	3	0.2736
9.7000	14.0000	0.182	14	2.553	1	−0.9508
9.7000	14.0000	0.182	14	2.553	1	−0.9508
9.7000	14.0000	0.182	14	2.553	0	−1.5630
9.7000	14.0000	0.182	14	2.553	2	−0.3386
9.7000	15.0000	0.182	15	2.735	4	0.7418
9.7000	15.0000	0.182	15	2.735	4	0.7418
9.7000	15.0000	0.182	15	2.735	3	0.1552
9.7000	15.0000	0.182	15	2.735	2	−0.4314
9.7000	16.0000	0.182	16	2.918	0	−1.6437
9.7000	16.0000	0.182	16	2.918	2	−0.5170
9.7000	16.0000	0.182	16	2.918	1	−1.0803
9.7000	16.0000	0.182	16	2.918	2	−0.5170
9.7000	17.0000	0.182	17	3.100	3	−0.0543
9.7000	17.0000	0.182	17	3.100	1	−1.1386
9.7000	17.0000	0.182	17	3.100	4	0.4879
9.7000	18.0000	0.182	18	3.282	3	−0.1476
9.7000	21.0000	0.182	21	3.830	4	0.0806
100.0000	11.0000	0.189	11	2.083	3	0.5323
100.0000	11.0000	0.189	11	2.083	1	−0.6282
100.0000	12.0000	0.189	12	2.272	0	−1.2357
100.0000	13.0000	0.189	13	2.461	0	−1.2604
100.0000	14.0000	0.189	14	2.651	2	−0.3149
100.0000	14.0000	0.189	14	2.651	3	0.1691
100.0000	14.0000	0.189	14	2.651	5	1.1369
100.0000	14.0000	0.189	14	2.651	2	−0.3149
100.0000	14.0000	0.189	14	2.651	6	1.6208
100.0000	14.0000	0.189	14	2.651	1	−0.7988
100.0000	14.0000	0.189	14	2.651	2	−0.3149
100.0000	15.0000	0.189	15	2.840	1	−0.8442
100.0000	15.0000	0.189	15	2.840	2	−0.3854
100.0000	15.0000	0.189	15	2.840	0	−1.3031
100.0000	15.0000	0.189	15	2.840	3	0.0734
100.0000	16.0000	0.189	16	3.029	4	0.4235
100.0000	16.0000	0.189	16	3.029	2	−0.4491
100.0000	17.0000	0.189	17	3.219	3	−0.0910
100.0000	17.0000	0.189	17	3.219	7	1.5729
100.0000	19.0000	0.189	19	3.597	10	2.4370
995.0000	7.0000	0.393	7	2.751	7	2.0149
995.0000	10.0000	0.393	10	3.930	2	−0.6684
995.0000	11.0000	0.393	11	4.323	3	−0.4205
995.0000	12.0000	0.393	12	4.716	0	−1.3852
995.0000	12.0000	0.393	12	4.716	6	0.3772
995.0000	13.0000	0.393	13	5.109	9	1.0623
995.0000	14.0000	0.393	14	5.502	4	−0.3831

995.0000	14.0000	0.393	14	5.502	0	−1.4032
995.0000	14.0000	0.393	14	5.502	2	−0.8932
995.0000	14.0000	0.393	14	5.502	10	1.1472
995.0000	15.0000	0.393	15	5.895	8	0.5037
995.0000	15.0000	0.393	15	5.895	3	−0.6928
995.0000	15.0000	0.393	15	5.895	9	0.7430
995.0000	15.0000	0.393	15	5.895	11	1.2216
995.0000	16.0000	0.393	16	6.288	15	1.9636
995.0000	16.0000	0.393	16	6.288	4	−0.5157
995.0000	16.0000	0.393	16	6.288	2	−0.9664
995.0000	17.0000	0.393	17	6.681	6	−0.1451
995.0000	17.0000	0.393	17	6.681	1	−1.2101
995.0000	17.0000	0.393	17	6.681	5	−0.3581
995.0000	20.0000	0.393	20	7.860	6	−0.3402

Observed Chi-square = 102.1763 Bootstrap Iterations per run = 10,000
p-value = 0.1423



BMR = 5% ER; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose, with fitted curve for the nested Rai and Van Ryzin model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from implantation through PND 4 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams {Ema, 2008, 787657}.

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The form of the probability function is:
Prob. = [1-exp(-Alpha-Beta*Dose^Rho)]*exp(-(Th1+Th2*Dose)*Rij),
where Rij is the litter specific covariate.

Restrict Power rho >= 1.

Benchmark Dose Computation
To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.425287
BMR = 5% ER
BMD = 315.585
BMDL at the 95% confidence level = 157.792

Parameter Estimates

Variable	Estimate	(Default) Initial parameter values
alpha	0.201085	0.201085
beta	7.58104 × 10−6	7.58104 × 10−6
rho	1.53267	1.53267
phi1	0.222343	0.222343
phi2	0.0213907	0.0213907
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Log-likelihood: −610.162 AIC: 1,234.32

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Dose	Lit.-Spec. Cov.	Litter Est._Prob.	Size	Scaled Expected	Observed	Residual
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0.0000	10.0000	0.182	10	1.822	4	1.0303
0.0000	11.0000	0.182	11	2.004	5	1.3037
0.0000	11.0000	0.182	11	2.004	0	−0.8718
0.0000	12.0000	0.182	12	2.186	1	−0.4778
0.0000	13.0000	0.182	13	2.368	0	−0.8885
0.0000	13.0000	0.182	13	2.368	3	0.2371
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0.0000	13.0000	0.182	13	2.368	0	−0.8885
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0.0000	14.0000	0.182	14	2.550	3	0.1579
0.0000	15.0000	0.182	15	2.732	15	4.0466
0.0000	15.0000	0.182	15	2.732	11	2.7271
0.0000	16.0000	0.182	16	2.915	4	0.3377
0.0000	16.0000	0.182	16	2.915	2	−0.2845
0.0000	16.0000	0.182	16	2.915	2	−0.2845
0.0000	16.0000	0.182	16	2.915	1	−0.5956
0.0000	16.0000	0.182	16	2.915	2	−0.2845
0.0000	16.0000	0.182	16	2.915	2	−0.2845
0.0000	17.0000	0.182	17	3.097	3	−0.0285
0.0000	17.0000	0.182	17	3.097	0	−0.9115
0.0000	17.0000	0.182	17	3.097	6	0.8546
0.0000	18.0000	0.182	18	3.279	1	−0.6365
9.7000	2.0000	0.182	2	0.365	2	2.9630

9.7000	12.0000	0.182	12	2.188	5	1.8912
9.7000	13.0000	0.182	13	2.371	3	0.4032
9.7000	13.0000	0.182	13	2.371	0	−1.5189
9.7000	13.0000	0.182	13	2.371	4	1.0439
9.7000	14.0000	0.182	14	2.553	3	0.2736
9.7000	14.0000	0.182	14	2.553	1	−0.9508
9.7000	14.0000	0.182	14	2.553	1	−0.9508
9.7000	14.0000	0.182	14	2.553	0	−1.5630
9.7000	14.0000	0.182	14	2.553	2	−0.3386
9.7000	15.0000	0.182	15	2.735	4	0.7418
9.7000	15.0000	0.182	15	2.735	4	0.7418
9.7000	15.0000	0.182	15	2.735	3	0.1552
9.7000	15.0000	0.182	15	2.735	2	−0.4314
9.7000	16.0000	0.182	16	2.918	0	−1.6437
9.7000	16.0000	0.182	16	2.918	2	−0.5170
9.7000	16.0000	0.182	16	2.918	1	−1.0803
9.7000	16.0000	0.182	16	2.918	2	−0.5170
9.7000	17.0000	0.182	17	3.100	3	−0.0543
9.7000	17.0000	0.182	17	3.100	1	−1.1386
9.7000	17.0000	0.182	17	3.100	4	0.4879
9.7000	18.0000	0.182	18	3.282	3	−0.1476
9.7000	21.0000	0.182	21	3.830	4	0.0806
100.0000	11.0000	0.189	11	2.083	3	0.5323
100.0000	11.0000	0.189	11	2.083	1	−0.6282
100.0000	12.0000	0.189	12	2.272	0	−1.2357
100.0000	13.0000	0.189	13	2.461	0	−1.2604
100.0000	14.0000	0.189	14	2.651	2	−0.3149
100.0000	14.0000	0.189	14	2.651	3	0.1691
100.0000	14.0000	0.189	14	2.651	5	1.1369
100.0000	14.0000	0.189	14	2.651	2	−0.3149
100.0000	14.0000	0.189	14	2.651	6	1.6208
100.0000	14.0000	0.189	14	2.651	1	−0.7988
100.0000	14.0000	0.189	14	2.651	2	−0.3149
100.0000	15.0000	0.189	15	2.840	1	−0.8442
100.0000	15.0000	0.189	15	2.840	2	−0.3854
100.0000	15.0000	0.189	15	2.840	0	−1.3031
100.0000	15.0000	0.189	15	2.840	3	0.0734
100.0000	16.0000	0.189	16	3.029	4	0.4235
100.0000	16.0000	0.189	16	3.029	2	−0.4491
100.0000	17.0000	0.189	17	3.219	3	−0.0910
100.0000	17.0000	0.189	17	3.219	7	1.5729
100.0000	19.0000	0.189	19	3.597	10	2.4370
995.0000	7.0000	0.393	7	2.751	7	2.0149
995.0000	10.0000	0.393	10	3.930	2	−0.6684
995.0000	11.0000	0.393	11	4.323	3	−0.4205
995.0000	12.0000	0.393	12	4.716	0	−1.3852
995.0000	12.0000	0.393	12	4.716	6	0.3772
995.0000	13.0000	0.393	13	5.109	9	1.0623
995.0000	14.0000	0.393	14	5.502	4	−0.3831
995.0000	14.0000	0.393	14	5.502	0	−1.4032
995.0000	14.0000	0.393	14	5.502	2	−0.8932
995.0000	14.0000	0.393	14	5.502	10	1.1472
995.0000	15.0000	0.393	15	5.895	8	0.5037
995.0000	15.0000	0.393	15	5.895	3	−0.6928

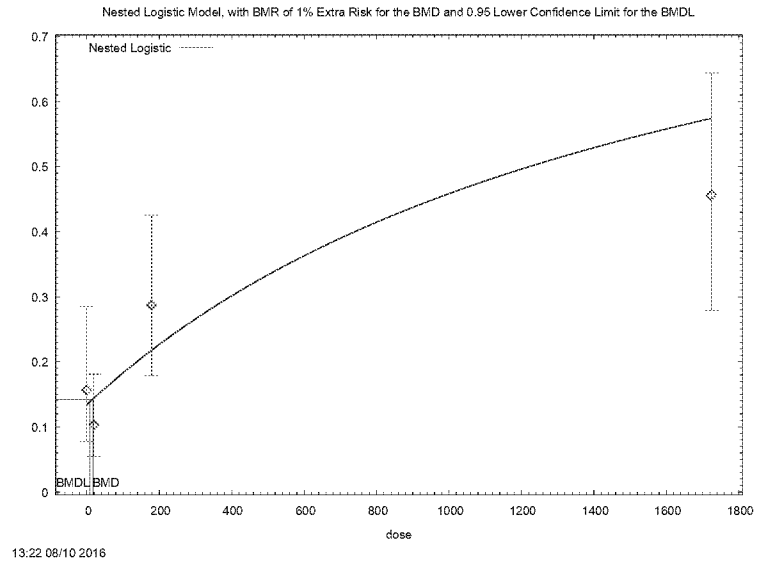
995.0000	15.0000	0.393	15	5.895	9	0.7430
995.0000	15.0000	0.393	15	5.895	11	1.2216
995.0000	16.0000	0.393	16	6.288	15	1.9636
995.0000	16.0000	0.393	16	6.288	4	−0.5157
995.0000	16.0000	0.393	16	6.288	2	−0.9664
995.0000	17.0000	0.393	17	6.681	6	−0.1451
995.0000	17.0000	0.393	17	6.681	1	−1.2101
995.0000	17.0000	0.393	17	6.681	5	−0.3581
995.0000	20.0000	0.393	20	7.860	6	−0.3402

Observed Chi-square = 102.1763 Bootstrap Iterations per run = 10,000
p-value = 0.1416

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for offspring loss from PND 4 through PND 21 in F2 offspring CRL Sprague-Dawley rats; lactational doses of F1 dams {Ema, 2008, 787657}; BMR = 1% ER and 5% ER

Model[a]	Goodness of Fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Litter-specific covariate = implantation size; intra-litter correlations estimated							Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the Nested Logistic model (litter-specific covariate not used; intra-litter correlations estimated) was selected based on lowest AIC (BMDLs differed by <3).
Nested Logistic	0.4417	561.04	20.4	10.1841	106.295	53.0644	
NCTR	0.4114	561.816	25.079	12.5395	127.994	63.997	
Rai and Van Ryzin	0.4056	564.38	25.8561	1.00024	131.96	5.9492	
Litter-specific covariate = implantation size; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	643.52	36.1762	22.5296	188.497	117.391	
NCTR	0.0000	650.146	33.8744	16.9372	172.883	86.4414	
Rai and Van Ryzin	0.0000	660.111	35.975	17.9875	183.603	91.8017	
Litter-specific covariate not used; intra-litter correlations estimated							
Nested Logistic	0.3944	559.472	16.9114	9.03491	88.1172	47.0766	
NCTR[b] Rai and Van Ryzin	0.4051	560.38	25.8566	12.9283	131.963	65.9814	
Litter-specific covariate not used; intra-litter correlations assumed to be zero							
Nested Logistic	0.0000	654.556	26.3666	18.3313	137.384	95.5159	
NCTR[b] Rai and Van Ryzin	0.0000	656.111	35.975	17.9875	183.603	91.8017	

[a]Because the individual animal data were available, the BMDS nested models were fitted, with the selected model in bold. For the selected model, the proportion of litters with scaled residuals above 2 in absolute value for doses 0, 19.6, 179, and 1,724 mg/kg-d were 2/22, 0/22, 2/20, and 0/20, respectively.
[b]With the litter-specific covariate not used, the NCTR and Rai and van Ryzin models yielded identical results.



BMR = 1% ER; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose, with fitted curve for the nested logistic model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from PND 4 through PND 21 in F2 offspring CRL Sprague-Dawley rats; lactational doses of F1 dams {Ema, 2008, 787657}.

Nested Logistic Model (Version: 2.20; Date: 04/27/2015)
The form of the probability function is:
Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/
[1+exp(-beta-theta2*Rij-rho*log(Dose))],
where Rij is the litter specific covariate.
Restrict Power rho >= 1.

Benchmark Dose Computation
To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.654762
BMR = 1% ER
BMD = 16.9114
BMDL at the 95% confidence level = 9.03491

Parameter Estimates

Variable	Estimate	(Default) Initial Parameter Values
alpha	0.133513	0.133513

beta	−7.42311	−7.42311
rho	1	1
phi1	0.229222	0.229222
phi2	0.152985	0.152985
phi3	0.247495	0.247495
phi4	0.586386	0.586386

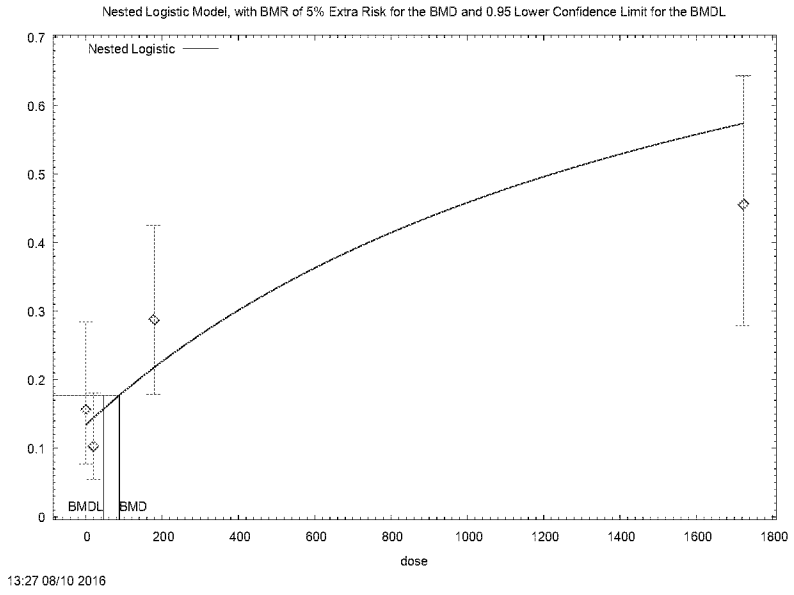
Log-likelihood: −273.736 AIC: 559.472

Goodness-of-Fit Table

Lit.-Spec. Dose	Lit.-Spec. Cov.	Litter Est. Prob.	Litter Size	Scaled Expected	Scaled Observed	Scaled Residual
0.0000	9.0000	0.134	6	0.801	0	−0.6563
0.0000	10.0000	0.134	6	0.801	1	0.1630
0.0000	11.0000	0.134	8	1.068	0	−0.6880
0.0000	11.0000	0.134	6	0.801	0	−0.6563
0.0000	12.0000	0.134	8	1.068	1	−0.0439
0.0000	13.0000	0.134	8	1.068	6	3.1766
0.0000	13.0000	0.134	8	1.068	0	−0.6880
0.0000	13.0000	0.134	8	1.068	3	1.2443
0.0000	13.0000	0.134	8	1.068	0	−0.6880
0.0000	14.0000	0.134	8	1.068	1	−0.0439
0.0000	14.0000	0.134	8	1.068	0	−0.6880
0.0000	15.0000	0.134	4	0.534	0	−0.6043
0.0000	16.0000	0.134	8	1.068	1	−0.0439
0.0000	16.0000	0.134	8	1.068	1	−0.0439
0.0000	16.0000	0.134	8	1.068	0	−0.6880
0.0000	16.0000	0.134	8	1.068	2	0.6002
0.0000	16.0000	0.134	8	1.068	1	−0.0439
0.0000	16.0000	0.134	8	1.068	4	1.8884
0.0000	17.0000	0.134	8	1.068	0	−0.6880
0.0000	17.0000	0.134	8	1.068	0	−0.6880
0.0000	17.0000	0.134	8	1.068	5	2.5325
0.0000	18.0000	0.134	8	1.068	0	−0.6880
19.6000	12.0000	0.144	7	1.005	2	0.7747
19.6000	13.0000	0.144	8	1.148	1	−0.1039
19.6000	13.0000	0.144	8	1.148	0	−0.8046
19.6000	13.0000	0.144	8	1.148	3	1.2975
19.6000	14.0000	0.144	8	1.148	2	0.5968
19.6000	14.0000	0.144	8	1.148	0	−0.8046
19.6000	14.0000	0.144	8	1.148	0	−0.8046
19.6000	14.0000	0.144	8	1.148	0	−0.8046
19.6000	14.0000	0.144	8	1.148	0	−0.8046
19.6000	15.0000	0.144	8	1.148	1	−0.1039
19.6000	15.0000	0.144	8	1.148	3	1.2975
19.6000	15.0000	0.144	8	1.148	0	−0.8046
19.6000	15.0000	0.144	8	1.148	1	−0.1039
19.6000	16.0000	0.144	8	1.148	0	−0.8046
19.6000	16.0000	0.144	8	1.148	0	−0.8046
19.6000	16.0000	0.144	8	1.148	0	−0.8046
19.6000	16.0000	0.144	8	1.148	0	−0.8046

19.6000	17.0000	0.144	8	1.148	1	−0.1039
19.6000	17.0000	0.144	8	1.148	0	−0.8046
19.6000	17.0000	0.144	8	1.148	3	1.2975
19.6000	18.0000	0.144	8	1.148	1	−0.1039
19.6000	21.0000	0.144	8	1.148	0	−0.8046
179.0000	11.0000	0.217	8	1.738	4	1.1735
179.0000	11.0000	0.217	8	1.738	2	0.1361
179.0000	12.0000	0.217	8	1.738	2	0.1361
179.0000	13.0000	0.217	8	1.738	0	−0.9013
179.0000	14.0000	0.217	8	1.738	2	0.1361
179.0000	14.0000	0.217	8	1.738	5	1.6922
179.0000	14.0000	0.217	8	1.738	3	0.6548
179.0000	14.0000	0.217	8	1.738	1	−0.3826
179.0000	14.0000	0.217	8	1.738	4	1.1735
179.0000	14.0000	0.217	8	1.738	1	−0.3826
179.0000	14.0000	0.217	8	1.738	6	2.2109
179.0000	15.0000	0.217	8	1.738	0	−0.9013
179.0000	15.0000	0.217	8	1.738	0	−0.9013
179.0000	15.0000	0.217	8	1.738	1	−0.3826
179.0000	15.0000	0.217	8	1.738	6	2.2109
179.0000	16.0000	0.217	8	1.738	0	−0.9013
179.0000	16.0000	0.217	8	1.738	4	1.1735
179.0000	17.0000	0.217	8	1.738	0	−0.9013
179.0000	17.0000	0.217	8	1.738	0	−0.9013
179.0000	19.0000	0.217	8	1.738	5	1.6922
1,724.0000	10.0000	0.573	8	4.585	4	−0.1850
1,724.0000	11.0000	0.573	8	4.585	2	−0.8178
1,724.0000	12.0000	0.573	8	4.585	1	−1.1341
1,724.0000	12.0000	0.573	6	3.439	0	−1.4313
1,724.0000	13.0000	0.573	4	2.292	1	−0.7865
1,724.0000	14.0000	0.573	8	4.585	8	1.0805
1,724.0000	14.0000	0.573	8	4.585	1	−1.1341
1,724.0000	14.0000	0.573	8	4.585	0	−1.4505
1,724.0000	14.0000	0.573	4	2.292	4	1.0392
1,724.0000	15.0000	0.573	7	4.012	3	−0.3637
1,724.0000	15.0000	0.573	8	4.585	0	−1.4505
1,724.0000	15.0000	0.573	6	3.439	6	1.0662
1,724.0000	15.0000	0.573	4	2.292	4	1.0392
1,724.0000	16.0000	0.573	1	0.573	1	0.8631
1,724.0000	16.0000	0.573	8	4.585	5	0.1313
1,724.0000	16.0000	0.573	8	4.585	0	−1.4505
1,724.0000	17.0000	0.573	8	4.585	3	−0.5014
1,724.0000	17.0000	0.573	8	4.585	8	1.0805
1,724.0000	17.0000	0.573	8	4.585	3	−0.5014
1,724.0000	20.0000	0.573	8	4.585	8	1.0805

Observed Chi-square = 86.7400 Bootstrap Iterations per run = 10,000
p-value = 0.3944



BMR = 5% ER; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of incidence rate by dose, with fitted curve for the nested logistic model where the litter specific covariate was not used and the intra-litter correlations were estimated, for incidence of offspring loss from PND 4 through PND 21 in F2 offspring CRL Sprague-Dawley rats; gestational doses of F1 dams {Ema, 2008, 787657}.

Nested Logistic Model (Version: 2.20; Date: 04/27/2015)
The form of the probability function is:
Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/
[1+exp(-beta-theta2*Rij-rho*log(Dose))],
where Rij is the litter specific covariate.
Restrict Power rho >= 1.

Benchmark Dose Computation
To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of all the data: 14.654762
BMR = 5% ER
BMD = 88.1172
BMDL at the 95% confidence level = 47.0766

Parameter Estimates

Variable	Estimate	(Default) Initial Parameter Values

alpha	0.133513	0.133513
beta	−7.42311	−7.42311
rho	1	1
phi1	0.229222	0.229222
phi2	0.152985	0.152985
phi3	0.247495	0.247495
phi4	0.586386	0.586386

Log-likelihood: −273.736 AIC: 559.472

Goodness-of-Fit Table

Lit.-Spec.		Litter			Scaled		
Dose	Cov.	Est. Prob.	Size	Expected	Observed	Residual
0.0000	9.0000	0.134	6	0.801	0	−0.6563
0.0000	10.0000	0.134	6	0.801	1	0.1630
0.0000	11.0000	0.134	8	1.068	0	−0.6880
0.0000	11.0000	0.134	6	0.801	0	−0.6563
0.0000	12.0000	0.134	8	1.068	1	−0.0439
0.0000	13.0000	0.134	8	1.068	6	3.1766
0.0000	13.0000	0.134	8	1.068	0	−0.6880
0.0000	13.0000	0.134	8	1.068	3	1.2443
0.0000	13.0000	0.134	8	1.068	0	−0.6880
0.0000	14.0000	0.134	8	1.068	1	−0.0439
0.0000	14.0000	0.134	8	1.068	0	−0.6880
0.0000	15.0000	0.134	4	0.534	0	−0.6043
0.0000	16.0000	0.134	8	1.068	1	−0.0439
0.0000	16.0000	0.134	8	1.068	1	−0.0439
0.0000	16.0000	0.134	8	1.068	0	−0.6880
0.0000	16.0000	0.134	8	1.068	2	0.6002
0.0000	16.0000	0.134	8	1.068	1	−0.0439
0.0000	16.0000	0.134	8	1.068	4	1.8884
0.0000	17.0000	0.134	8	1.068	0	−0.6880
0.0000	17.0000	0.134	8	1.068	0	−0.6880
0.0000	17.0000	0.134	8	1.068	5	2.5325
0.0000	18.0000	0.134	8	1.068	0	−0.6880
19.6000	12.0000	0.144	7	1.005	2	0.7747
19.6000	13.0000	0.144	8	1.148	1	−0.1039
19.6000	13.0000	0.144	8	1.148	0	−0.8046
19.6000	13.0000	0.144	8	1.148	3	1.2975
19.6000	14.0000	0.144	8	1.148	2	0.5968
19.6000	14.0000	0.144	8	1.148	0	−0.8046
19.6000	14.0000	0.144	8	1.148	0	−0.8046
19.6000	14.0000	0.144	8	1.148	0	−0.8046
19.6000	14.0000	0.144	8	1.148	0	−0.8046
19.6000	15.0000	0.144	8	1.148	1	−0.1039
19.6000	15.0000	0.144	8	1.148	3	1.2975
19.6000	15.0000	0.144	8	1.148	0	−0.8046
19.6000	15.0000	0.144	8	1.148	1	−0.1039
19.6000	16.0000	0.144	8	1.148	0	−0.8046
19.6000	16.0000	0.144	8	1.148	0	−0.8046

19.6000	16.0000	0.144	8	1.148	0	−0.8046
19.6000	16.0000	0.144	8	1.148	0	−0.8046
19.6000	17.0000	0.144	8	1.148	1	−0.1039
19.6000	17.0000	0.144	8	1.148	0	−0.8046
19.6000	17.0000	0.144	8	1.148	3	1.2975
19.6000	18.0000	0.144	8	1.148	1	−0.1039
19.6000	21.0000	0.144	8	1.148	0	−0.8046
179.0000	11.0000	0.217	8	1.738	4	1.1735
179.0000	11.0000	0.217	8	1.738	2	0.1361
179.0000	12.0000	0.217	8	1.738	2	0.1361
179.0000	13.0000	0.217	8	1.738	0	−0.9013
179.0000	14.0000	0.217	8	1.738	2	0.1361
179.0000	14.0000	0.217	8	1.738	5	1.6922
179.0000	14.0000	0.217	8	1.738	3	0.6548
179.0000	14.0000	0.217	8	1.738	1	−0.3826
179.0000	14.0000	0.217	8	1.738	4	1.1735
179.0000	14.0000	0.217	8	1.738	1	−0.3826
179.0000	14.0000	0.217	8	1.738	6	2.2109
179.0000	15.0000	0.217	8	1.738	0	−0.9013
179.0000	15.0000	0.217	8	1.738	0	−0.9013
179.0000	15.0000	0.217	8	1.738	1	−0.3826
179.0000	15.0000	0.217	8	1.738	6	2.2109
179.0000	16.0000	0.217	8	1.738	0	−0.9013
179.0000	16.0000	0.217	8	1.738	4	1.1735
179.0000	17.0000	0.217	8	1.738	0	−0.9013
179.0000	17.0000	0.217	8	1.738	0	−0.9013
179.0000	19.0000	0.217	8	1.738	5	1.6922
1,724.0000	10.0000	0.573	8	4.585	4	−0.1850
1,724.0000	11.0000	0.573	8	4.585	2	−0.8178
1,724.0000	12.0000	0.573	8	4.585	1	−1.1341
1,724.0000	12.0000	0.573	6	3.439	0	−1.4313
1,724.0000	13.0000	0.573	4	2.292	1	−0.7865
1,724.0000	14.0000	0.573	8	4.585	8	1.0805
1,724.0000	14.0000	0.573	8	4.585	1	−1.1341
1,724.0000	14.0000	0.573	8	4.585	0	−1.4505
1,724.0000	14.0000	0.573	4	2.292	4	1.0392
1,724.0000	15.0000	0.573	7	4.012	3	−0.3637
1,724.0000	15.0000	0.573	8	4.585	0	−1.4505
1,724.0000	15.0000	0.573	6	3.439	6	1.0662
1,724.0000	15.0000	0.573	4	2.292	4	1.0392
1,724.0000	16.0000	0.573	1	0.573	1	0.8631
1,724.0000	16.0000	0.573	8	4.585	5	0.1313
1,724.0000	16.0000	0.573	8	4.585	0	−1.4505
1,724.0000	17.0000	0.573	8	4.585	3	−0.5014
1,724.0000	17.0000	0.573	8	4.585	8	1.0805
1,724.0000	17.0000	0.573	8	4.585	3	−0.5014
1,724.0000	20.0000	0.573	8	4.585	8	1.0805

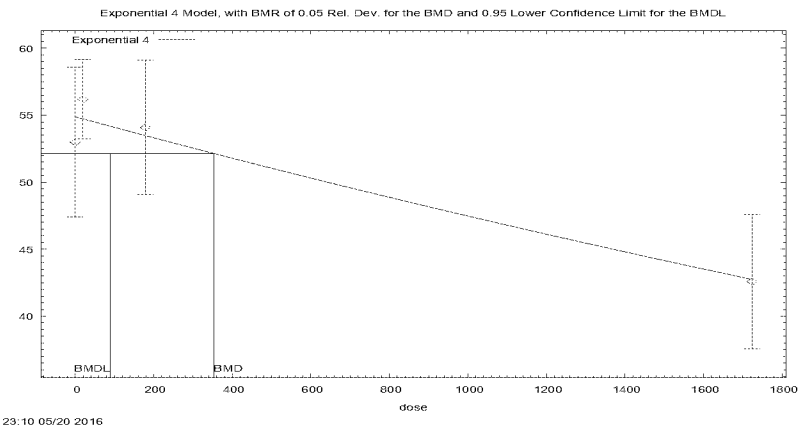
Observed Chi-square = 86.7400 Bootstrap Iterations per run = 10,000
p-value = 0.4003

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose({Ema, 2008, 787657};

BMR = 5% RD from control mean, 10% RD from control mean, 0.5 SD change from control mean, and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{5RD} (mg/kg-d)	$BMDL_{5RD}$ (mg/kg-d)	BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2)	0.486	420.90	354	240	727	494	Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the Exponential M4 constant variance model was selected based on lowest BMDL (BMDLs differed by >3).
Exponential (M3)	0.266	422.69	651	244	1016	500	
Exponential (M4)	0.486	420.90	354	89.6	727	206	
Exponential (M5)	N/A[b]	424.68	230	94.0	258	181	
Hill	N/A[b]	424.68	230	89.2	264	errore	
Power	0.266	422.69	676	282	1,049	565	
Polynomial 3° Polynomial 2°	0.264	422.70	817	282	1,161	564	
Linear	0.497	420.85	389	280	779	560	
Model[a]	Goodness of fit		$BMD_{0.5SD}$ (mg/kg-d)	$BMDL_{0.5SD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	
	p-value	AIC					
Exponential (M2)	0.486	420.90	634	419	1,332	879	
Exponential (M3)	0.266	422.69	937	425	1,483	891	
Exponential (M4)	0.486	420.90	634	172	1,332	468	
Exponential (M5)	N/Ab	424.68	252	176	296	189	
Hill	N/Ab	424.68	256	176	324	error[c]	
Power	0.266	422.69	969	482	1,503	965	
Polynomial 3° Polynomial 2°	0.264	422.70	1,091	482	1,549	964	
Linear	0.497	420.85	684	478	1,368	956	

[a]Constant variance case presented (BMDS Test 2 p-value = 0.0278), selected model in bold; scaled residuals for selected model for doses 0, 19.6, 179, and 1,724 mg/kg-day were −0.92, 0.71, 0.27, and −0.06, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.



BMR = 5% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 5% RD
BMD = 353.728
BMDL at the 95% confidence level = 89.5935

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	53	54.89	12.6	9.64	−0.9187

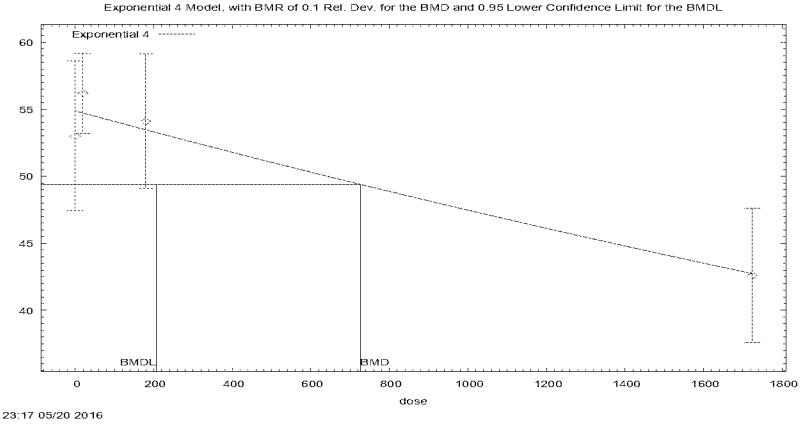
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	−0.0551

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−206.7258	5	423.4517
A2	−202.1665	8	420.333
A3	−206.7258	5	423.4517
R	−214.7267	2	433.4535
4	−207.4482	3	420.8963

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 726.585
BMDL at the 95% confidence level = 206.377

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

Table of Data and Estimated Values of Interest

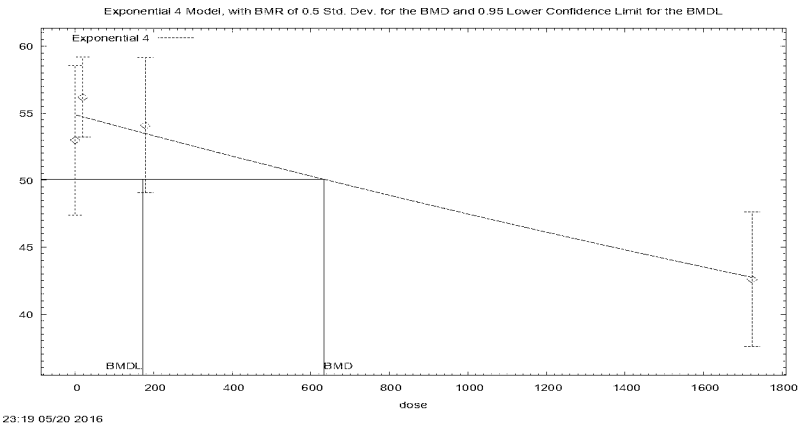
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	53	54.89	12.6	9.64	−0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	−0.0551

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−206.7258	5	423.4517
A2	−202.1665	8	420.333
A3	−206.7258	5	423.4517
R	−214.7267	2	433.4535
4	−207.4482	3	420.8963

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856



BMR = 0.5 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 50% Estimated SDs from control
BMD = 633.879
BMDL at the 95% confidence level = 171.599

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals

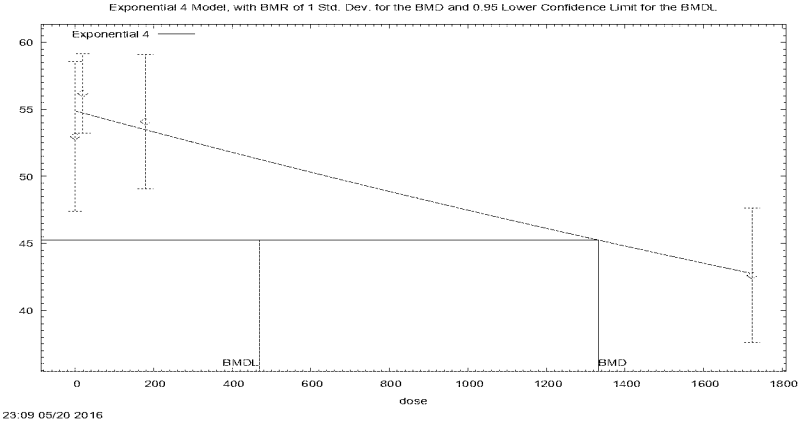
0	22	53	54.89	12.6	9.64	−0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	−0.0551

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−206.7258	5	423.4517
A2	−202.1665	8	420.333
A3	−206.7258	5	423.4517
R	−214.7267	2	433.4535
4	−207.4482	3	420.8963

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Exponential (M4) model with constant variance for pup weight during lactation in F2 male offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Exponential Model (Version: 1.10; Date: 01/12/2015)
The form of the response function is: Y[dose] = a * [c-(c-1) * exp(-b * dose)]
A constant variance model is fit

Benchmark Dose Computation
BMR = 1.0000 Estimated SDs from control
BMD = 1331.98
BMDL at the 95% confidence level = 468.431

Parameter Estimates

Variable	Estimate	Default initial parameter values
lnalpha	4.53195	4.51269
rho	N/A	0
a	54.8883	59.01
b	0.000145008	0.00128594
c	0	0.687535
d	N/A	1

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	22	53	54.89	12.6	9.64	−0.9187
19.6	22	56.2	54.73	6.7	9.64	0.714
179	18	54.1	53.48	10.1	9.64	0.272
1,724	13	42.6	42.75	8.3	9.64	−0.0551

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−206.7258	5	423.4517
A2	−202.1665	8	420.333
A3	−206.7258	5	423.4517
R	−214.7267	2	433.4535
4	−207.4482	3	420.8963

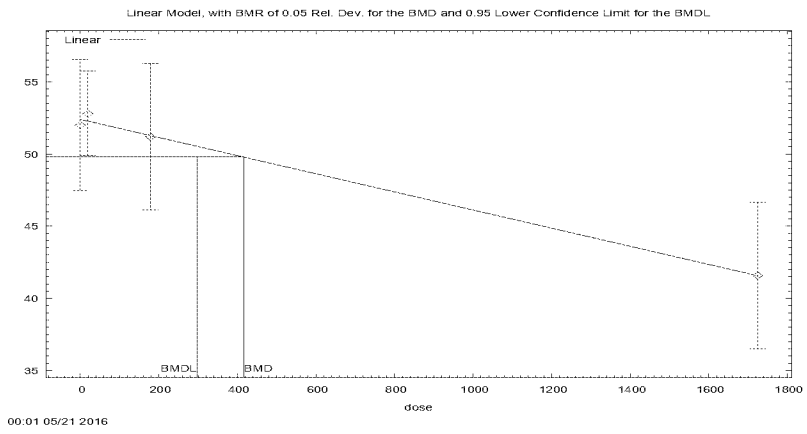
Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	25.12	6	0.0003244
Test 2	9.119	3	0.02775
Test 3	9.119	3	0.02775
Test 6a	1.445	2	0.4856

Table [STYLEREF 1 \s]-[SEQ Table * ARABIC \s 1]. Summary of BMD modeling results for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}; BMR = 5% RD from control mean, 10% RD from control mean, 0.5 SD change from control mean and 1 SD change from control mean

Model[a]	Goodness of fit		BMD_{5RD} (mg/kg-d)	$BMDL_{5RD}$ (mg/kg-d)	BMD_{10RD} (mg/kg-d)	$BMDL_{10RD}$ (mg/kg-d)	Basis for model selection
	p-value	AIC					
Exponential (M2)	0.942	413.8640	381	257	783	528	Of the models that provided an adequate fit, a valid BMDL estimate and BMD/BMDL <5, the Linear constant variance model was selected based on lowest AIC (BMDLs differed by <3).
Exponential (M3)	0.732	415.86	411	257	815	529	
Exponential (M4)	0.729	415.86	381	257	783	528	
Exponential (M5)	N/A[b]	417.83	201	76.5	225	179	
Hill	N/A[b]	417.83	203	67.7	235	error[c]	
Power	0.729	415.86	423	297	840	594	
Polynomial 3°[c] Polynomial 2°[d] Linear	0.942	413.8637	417	297	834	594	
Model[a]	Goodness of fit		$BMD_{0.5SD}$ (mg/kg-d)	$BMDL_{0.5SD}$ (mg/kg-d)	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	
	p-value	AIC					
Exponential (M2)	0.942	413.864	657	432	1378	903	
Exponential (M3)	0.732	415.86	690	432	1397	903	
Exponential (M4)	0.729	415.86	657	432	1378	903	
Exponential (M5)	N/Ab	417.83	219	140	256	188	
Hill	N/Ab	417.83	226	133	291	error$_c$	
Power	0.729	415.86	712	489	1,416	978	
Polynomial 3° Polynomial 2° Linear	0.942	413.8637	706	489	1,412	978	

[a]Constant variance case presented (BMDS Test 2 p-value = 0.133), selected model in bold; scaled residuals for selected model for doses 0, 19.6, 179, and 1,724 mg/kg-day were −0.22, 0.26, −0.05, and 0, respectively.
[b]No available degrees of freedom to calculate a goodness-of-fit value.
[c]BMD or BMDL computation failed for this model.



BMR = 5% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Polynomial Model (Version: 2.20; Date: 10/22/2014)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose
A constant variance model is fit

Benchmark Dose Computation
BMR = 5% RD
BMD = 417.145
BMDL at the 95% confidence level = 296.948

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	−0.00628402	−0.00627654

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	−0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	−0.0514

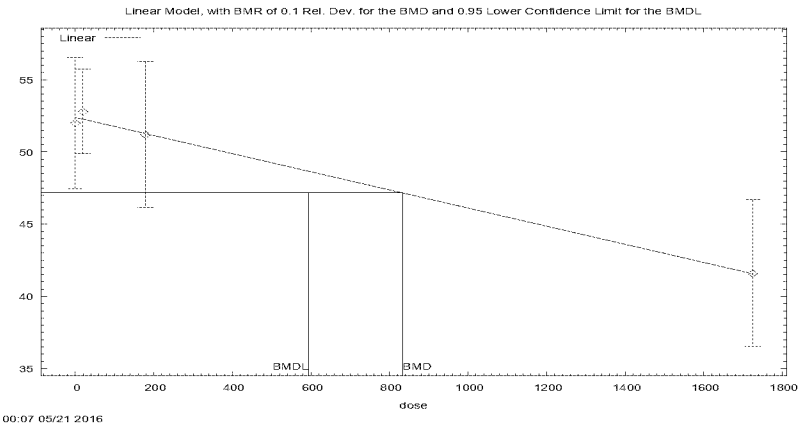
1,724	13	41.6	41.6	8.4	8.88	0.00274

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−203.871816	5	417.743631
A2	−201.070527	8	418.141053
A3	−203.871816	5	417.743631
fitted	−203.931869	3	413.863738
R	−210.813685	2	425.627371

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417



BMR = 10% RD from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Polynomial Model (Version: 2.20; Date: 10/22/2014)

The form of the response function is: Y[dose] = beta_0 + beta_1*dose

A constant variance model is fit

Benchmark Dose Computation
BMR = 10% RD
BMD = 834.289
BMDL at the 95% confidence level = 593.896

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	−0.00628402	−0.00627654

Table of Data and Estimated Values of Interest

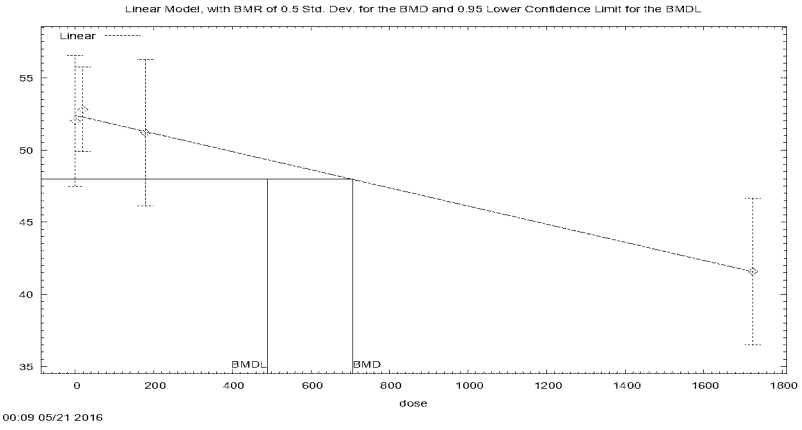
Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	−0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	−0.0514
1,724	13	41.6	41.6	8.4	8.88	0.00274

Likelihoods of Interest

Model	Log (likelihood)	Number of parameters	AIC
A1	−203.871816	5	417.743631
A2	−201.070527	8	418.141053
A3	−203.871816	5	417.743631
fitted	−203.931869	3	413.863738
R	−210.813685	2	425.627371

Tests of Interest

Test	−2*log (likelihood ratio)	Test df	p-value
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417



BMR = 0.5 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Polynomial Model (Version: 2.20; Date: 10/22/2014)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose
A constant variance model is fit

Benchmark Dose Computation
BMR = 50% Estimated SDs from the control mean
BMD = 706.21
BMDL at the 95% confidence level = 488.985

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	−0.00628402	−0.00627654

Table of Data and Estimated Values of Interest

Dose	N	Observed mean	Estimated mean	Observed SD	Estimated SD	Scaled residuals
0	21	52	52.4	10	8.88	−0.22
19.6	22	52.8	52.3	6.6	8.88	0.262
179	20	51.2	51.3	10.8	8.88	−0.0514

1,724	13	41.6	41.6	8.4	8.88	0.00274

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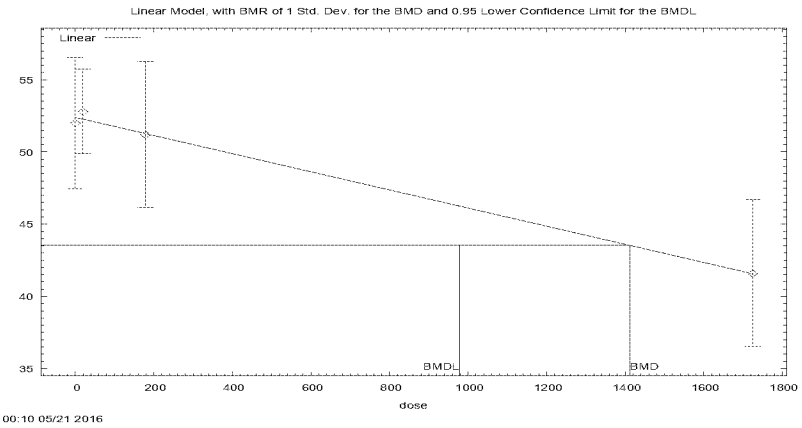
Test 1	19.4863	6	0.003416
Test 2	5.60258	3	0.1326
Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417



BMR = 1 SD change from control mean; dose shown in mg/kg-day.

Figure [STYLEREF 1 \s]-[SEQ Figure * ARABIC \s 1]. Plot of mean response by dose with fitted curve for Linear model with constant variance for pup weight during lactation in F2 female offspring CRL Sprague-Dawley rats (PND 21) exposed to HBCD by diet for 3 weeks, lactational dose {Ema, 2008, 787657}.

Polynomial Model (Version: 2.20; Date: 10/22/2014)
The form of the response function is: Y[dose] = beta_0 + beta_1*dose

A constant variance model is fit

Benchmark Dose Computation
BMR = 1 Estimated SDs from the control mean
BMD = 1412.42
BMDL at the 95% confidence level = 977.97

Parameter Estimates

Variable	Estimate	Default initial parameter values
alpha	78.7776	83.0228
rho	N/A	0
beta_0	52.4269	52.4168
beta_1	−0.00628402	−0.00627654

Table of Data and Estimated Values of Interest

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fitted	−203.931869	3	413.863738
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Test 3	5.60258	3	0.1326
Test 4	0.120106	2	0.9417